

REMARKS

The present application relates to inbred maize line PH94T. Claims 1-36 are pending in the present application. No new matter has been added by way of amendment. Applicant respectfully requests consideration of the claims in view of the following remarks.

Detailed Action

Applicant acknowledges that the terminal disclaimer filed on April 19, 2006 has been accepted and recorded.

Rejections Under 35 U.S.C. § 112, First Paragraph

Claims 1-14 and 17-36 stand rejected under 35 U.S.C. § 112, first paragraph, as failing to comply with the written description requirement. The Examiner asserts that the claims contain subject matter which was not described in the specification in such a way as to reasonably convey to one skilled in the relevant art that the inventor, at the time the application was filed, had possession of the claimed invention. The Examiner states the decision in *Ex parte Carlson* "is not persuasive because the decision by the Board is unpublished opinion ... and cannot be cited as precedent". In addition, the Examiner states the rejection is repeated for claims for the reasons of record set forth in the Office Action of December 19, 2005. The Examiner further states that the "development and mapping of SSR markers having no known linked trait would not provide adequate written description for hybrid seed/plants produced from inbred parent lines with unknown genetic complement". See Office Action, p. 2-3.

Applicant respectfully traverses this rejection. Firstly, while Applicant recognizes that *Ex parte Carlson* is not binding precedent, Applicant respectfully urges that the written description requirement for one substantively similar case is indicative of an appropriate standard for the instant case.

The written description requirement may be fulfilled by identifying a structural feature which is present in each member of a claimed genus. *Regents of University of California*, 119 F.3d at 1568, 43 U.S.P.Q.2d at 1406 (teaching that claims may satisfy the written description requirement where they disclose "structural features commonly possessed by members of the genus that distinguish them from others.") The maize seeds, plants and plant parts of claims 1-14 and 17-36 all share the same genetic component and/or cells received from inbred parent

PH94T. Applicant previously presented a diagram, in the Amendment submitted April 18, 2005 as Exhibit 1, which is a visual representation of the fact that most of the cells in a maize inbred will have two essentially duplicate sets of ten chromosomes. (For illustrative purposes the ten chromosomes were represented by three rectangles in the Exhibits). In order to produce an F1 hybrid, the inbred will produce a haploid cell, such as pollen or an ovule. These haploid cells receive one copy of the inbred's duplicate sets of chromosomes. Accordingly, the F1 hybrid seed receives one complete set of chromosomes from the inbred parent, regardless of whether the inbred is used as the male or female parent of the F1 hybrid. (*See Previously submitted Exhibits 2 and 3*).

As known by one skilled in the art, the reason an F1 hybrid produced from an inbred maize line will always receive one complete set of chromosomes from the inbred parent is because the genome of a maize inbred line is homozygous. This homozygosity is a consequence of self pollination that occurs during the inbreeding process. As described in the Specification:

The inbred has shown uniformity and stability within the limits of environmental influence for all the traits as described in the Variety Description Information (Table 1) that follows. The inbred has been self-pollinated and ear-rowed a sufficient number of generations with careful attention paid to uniformity of plant type to ensure the homozygosity and phenotypic stability necessary to use in commercial production. The line has been increased both by hand and in isolated fields with continued observation for uniformity. No variant traits have been observed or are expected in PH94T. (Specification, p. 26, ll. 17-24).

Applicant's invention relates to hybrid seed and plants which are produced by crossing inbred maize line PH94T with another maize plant. As described *infra*, each member of the genus of hybrids which has PH94T as a parent and which is encompassed by claims 1-14 and 17-36 contains the chromosomes of inbred line PH94T

Applicant reiterates that at least 95% of the alleles of inbred line PH94T disclosed in the SSR profile of Table 4 is an identifying physical characteristic that describes the genus of minor variants of inbred line PH94T, including, but not limited to, single locus conversions produced through transformation or introgression. The SSR profile of PH94T is disclosed for numerous markers distributed throughout the genome as indicated by the Bin number of the marker, which denotes the marker location. A plant comprising 95% of the alleles of PH94T as disclosed in Table 4 would be produced, for example, by repeated backcrossing to PH94T. A backcross

conversion of PH94T as claimed in the instant application is described as comprising 95% of the alleles disclosed in Table 4.

The set of chromosomes of PH94T that will be retained in a hybrid made with PH94T can be obtained from the deposited seed and are disclosed in the SSR profile which one of ordinary skill in the art has access to. *See* specification, Table 4, pp. 70-73. These molecular markers allow one of ordinary skill in the art to distinguish a maize plant containing a set of chromosomes of PH94T from other maize plants

It is undisputed that fingerprinting with molecular markers is widely used for characterizing germplasm. Specifically, SSR profiles are known and can be practiced by one of ordinary skill in the art in maize breeding. One of ordinary skill has been enabled by the deposit to make and use minor variants of inbred corn line PH94T, and one of ordinary skill in the art uses SSR markers to characterize backcross conversions of an inbred. Applicant has claimed in the manner used by those of ordinary skill in the art to characterize backcross conversions.

Applicant further asserts that molecular marker methods are known to one ordinarily skilled in the art and the SSR profile of PH94T can be obtained from the deposit, but notwithstanding, Applicant has also provided the SSR profile of PH94T in the application. *See* specification, Table 4, pp. 70-73. Applicant reiterates that according to *Enzo*, the deposit of a material in a public depository is an adequate description of that material for purposes of the written description requirement. *Enzo Biochem, Inc.*, 296 F.3d at 1325, 63 U.S.P.Q.2d at 1613. In addition, *Regents of University of California*, 119 F.3d at 1568, 43 U.S.P.Q.2d at 1406, teaches that claims may satisfy the written description requirement where they disclose "structural features commonly possessed by members of the genus that distinguish them from others." The Board of Patent Appeals & Interferences has also confirmed the sufficiency of a deposit for seed and plants in the case of *Ex Parte C*, 1992 WL 515817 p. * 5, 27 U.S.P.Q.2d 1492, 1496 (B.P.A.I. 1992), where it stated that "[t]he claimed soybean is described in the specification to the extent that there is no question that appellant was in possession of the invention as of the time the instant application was filed. Because seed is to be deposited in a public depository, the specification is enabling and sets forth the best mode of carrying out the invention." Consistent with this principal, the Board of Patent Appeals & Interferences, in a case involving the written description requirement as applied to seed and plants, stated "[i]f in making the latter comment the examiner is requiring appellants to have reduced to practice each possible

plant within the scope of the claims, such a position is legally incorrect. The specification need only teach one skilled in the art how to make and use the claimed invention. How the specification does so, whether by way of the written word or actual examples, is of no moment." *Ex parte Gerardu C.M. Bentvelsen et al.*, 2001 WL 1197757, p. *2 (B.P.A.I. 2001).

The Applicant further asserts those of skill in the art utilize molecular markers, such as SSR's, to characterize plant genomes. As Applicant's clearly teach in the specification:

To accomplish this goal, the maize breeder must select and develop superior inbred parental lines for producing hybrids. This requires identification and selection of genetically unique individuals that occur in a segregating population. The segregating population is the result of a combination of crossover events plus the independent assortment of specific combinations of alleles at many gene loci that results in specific genotypes. *See specification*, p. 10, ll. 15-21.

Further, Applicant teaches:

In addition to phenotypic observations, the genotype of a plant can also be examined. A plant's genotype can be used to identify plants of the same variety or a related variety. For example, the genotype can be used to determine the pedigree of a plant. There are many laboratory-based techniques available for the analysis, comparison and characterization of plant genotype; among these are Isozyme Electrophoresis, Restriction Fragment Length Polymorphisms (RFLPs), Randomly Amplified Polymorphic DNAs (RAPDs), Arbitrarily Primed Polymerase Chain Reaction (AP-PCR), DNA Amplification Fingerprinting (DAF), Sequence Characterized Amplified Regions (SCARs), Amplified Fragment Length Polymorphisms (AFLPs), Simple Sequence Repeats (SSRs) which are also referred to as Microsatellites, and Single Nucleotide Polymorphisms (SNPs). *See specification*, p. 25, ll. 1-11.

Applicant also teaches how the claimed backcross trait conversions are "routinely used and have a very high rate of success". *See specification*, p. 37, ll. 14-15. Those plants and plant parts that are developed substantially benefiting from the use of inbred maize line PH94T "comprising a single gene conversion, transgene, or genetic sterility factor, may be identified by having a molecular marker profile with a high percent identity to PH94T". *See specification*, p. 73, ll. 30-32.

The use of molecular marker profiles by those of ordinary skill in the art in backcrossing is also clearly supported by the scientific literature. For example, see Ragot, M. *et al.* (1995) Marker-assisted backcrossing: a practical example, in *Techniques et Utilisations des Marqueurs Moleculaires (Les Colloques*, Vol. 72, pp. 45-56 (attached as Appendix 1), and Openshaw *et al.*,

(1994) Marker-assisted Selection in Backcross Breeding, Analysis of Molecular Marker Data, pp. 41-43 (attached as Appendix 2). Specifically, Ragot *et al.* notes that "spectacular" progress toward the recurrent parent genotype was obtained with 61 RFLP markers. Ragot *et al.* also concludes that "recovery of the recurrent parent genotype could proceed even faster than in the experiment described herein, should the appropriate protocol and resources (population size, number and position of markers) be allocated." In the case at issue, over 125 markers have been provided. SSR markers have been demonstrated to be at least as reliable, if not more so, than RFLP markers. See J.S.C. Smith *et al.*, An Evaluation of the Utility of SSR Loci as Molecular Markers in Maize (*Zea Mays* L.): Comparisons with Data from RFLPS and Pedigree, Theor. App. Genet. 95:163-173 (1997) (attached as Appendix 3). Accordingly, it is clear that at least 95% identity based on over 125 SSR markers is more than sufficient to characterize the claimed backcross conversions of PH94T to one of ordinary skill in the art.

Thus, SSR profiles are known and can be practiced by one of ordinary skill in the art. One of ordinary skill has been enabled by the deposit to make and use backcross conversions of inbred corn line PH94T, and one of ordinary skill in the art uses molecular markers to characterize backcross conversions of an inbred line. Applicant has claimed in the manner used by those of ordinary skill in the art to characterize backcross conversions, and 95% identity based on over 125 SSR markers is more than sufficient to characterize such conversions.

The state of the art is such that it is routine to produce backcross conversions, a statement supporting by Ragot *et al.*, Openshaw *et al.*, as well as basic textbooks on plant breeding. For example, See Hallauer *et al.*, "Corn Breeding", Corn and Corn Improvement, No. 18, p. 472 (1988) and Poehlman *et al.*, Breeding Field Crop, 4th Ed., Iowa State University Press, Ames, IA, p. 334 (1995). Specifically, Ragot *et al.* states in the first sentence of the summary "[t]hat molecular markers allow fast recovery of recurrent parent genotype in backcross programs is undisputed", and, in the first sentence of the introduction, "[b]ackcrossing has been a common breeding practice for as long as elite germplasm has been available." The Applicant's specification teaches that molecular markers of PH94T can also be used to "reduce the number of crosses back to the recurrent parent needed in a backcrossing program". See specification, p. 7, ll. 27-28. In fact, many of the transgenic corn lines currently being commercialized are the result of a backcross conversion of a novel inbred, such as PH94T.

Furthermore, Applicant reiterates that the written description requirement of § 112, first paragraph has been fulfilled by depositing seeds of PH94T in a public depository and by referencing the deposit in the specification. *See* specification, p. 76; *see also Enzo Biochem, Inc. v. Gen-Probe Inc.*, 323 F.3d 956, 965, 63 U.S.P.Q.2d 1609, 1613 (Fed. Cir. 2002) (stating that the written description requirement of § 112, ¶ 1 may be fulfilled by depositing material in a public depository, where the deposited material is not accessible in writing, and where reference to the deposit is made in the specification). This deposit not only describes inbred maize line PH94T but also the hybrid maize plants, plant parts, and seeds grown in claims 1-14 and 17-36. Applicant reiterates the Board of Patent Appeals and Interferences determined that where claims to an inbred maize plant satisfied the written description requirement, claims to the F1 hybrid seed and plants with the inbred maize plant as a parent also satisfied the written description requirement and thus the written description requirement for one substantively similar case is indicative of an appropriate standard for the instant case. *See Ex parte Carlson* (B.P.A.I. 2005).

In addition, Applicant asserts the written description requirement does not mandate a description by phenotype. At its foundation, the written description requirement serves an evidentiary function of making certain that the Applicant's are in possession of a specific characteristic that identifies their claimed invention. The molecular marker data provided by Applicant's serves this purpose. The other inbred is not the point of patentability, nor is it what is being claimed. Rather, the claim is drawn precisely to what is described, an F1 hybrid with the identifiable and unique molecular profile of PH94T.

Accordingly, Applicant submits that claims 1-14 and 17-36 are described. In light of the above amendments and remarks, Applicant respectfully requests reconsideration and withdrawal of the rejections under 35 U.S.C. § 112, first paragraph.

Rejections Under 35 U.S.C. §§ 102(b)/103(a)

Claims 1-14 and 17-36 are rejected under 35 U.S.C. § 102(b) as being anticipated by or, in the alternative, under 35 U.S.C. § 103(a) as obvious over Fullerton et al., (U.S. Patent 6,169,234). The Examiner states that "given all these similarities between the prior art hybrid and the inbred line PH94T, the prior art plant and seed would comprise at least on set of chromosomes of the inbred line PH94T ... and the claimed seed/plant and those of the prior art are indistinguishable". *See* Office Action, pp. 3-5.

Applicant respectfully traverses this rejection. The Applicant would like to point out that the inventions inbred maize line PH94T and hybrid maize plant and seed 36B08 are not the same inventions. Fullerton et al. does not disclose each of the limitations of claims 1-14 and 17-36. Nor are their differences minor morphological variations. Applicant submits that the claimed plant cannot be anticipated by Fullerton et al. as inbred maize line PH94T and hybrid maize plant and seed 36B08 are not the same as it possesses a unique combination of traits which confers a unique combination of genetics. Moreover, Applicant claims a method of making a plant which did not previously exist. Maize inbred line PH94T has not previously existed as it is the result of crossing two inbred maize lines PHME2 and PH1K2 (see Appendix 4, PVP Certificate No. 200200192). In contrast Fullerton et al. is a hybrid plant that is produced by crossing two inbred maize lines GE515721 with ATCC Deposit PTA-1306 and GE501400 with ATCC Deposit PTA-1282.

Furthermore, when looking at the tables of both inventions, hybrids created using PH94T as one of the parents are clearly not anticipated by hybrids made using 36B08 as one of the parents. The inventions PH94T and 36B08 differ for various traits that are not minor. For example:

CHARACTERISTICS	<u>Inbred PH94T</u>	<u>Hybrid 36B08</u>
Comparative Relative Maturity	85	102
Rating System		
Ear Height (cm)	77.0	104.0
Pollen Shed	5.3	7.0
Hard Endosperm Color	Pink-Orange	Yellow
Anthracnose Stalk Rot	9	6
Rate from 1 (most susceptible) to 9 (most resistant)		
Staygreen	4	6

This comparison clearly shows that PH94T does not exhibit the characteristics of hybrid 36B08. In addition, it is vital to note that the cited prior art is a hybrid and not an inbred as in the present invention and one of ordinary skill in the art would know the major differences between

a hybrid and inbred. The aforementioned examples all illustrate that there are large differences between PH94T and 36B08. The examples listed are not exhaustive but they do give ample evidence that the inventions are not the same. Furthermore, when looking at the tables of both inventions, plants created using PH94T as one of the parents are clearly not anticipated by hybrids made using 36B08 as one of the parents.

When looking at a maize plant it would be possible to find many traits that are similar between varieties such as the color of flowers or growth habit. However, to say there are similarities in phenotype between two varieties is not the same as saying that the two varieties have the same morphological and physiological characteristics as a whole, or that one is an obvious variant of the other.

As described *supra*, inbred maize line PH94T does not exhibit the same characteristics as hybrid maize plant and seed 36B08. The Examiner has not provided any reference that may be combined with 36B08 to arrive at the present invention. The Examiner has not provided a single reference with all elements of the claimed invention, nor a reference that could be combined with the Fullerton et al. patent to produce PH94T. Applicant respectfully asserts that a prima facie case of obviousness has not been made, and reconsideration is respectfully requested. Thus, Applicant submits that the claimed plant cannot be rendered obvious over Fullerton et al.. Inbred PH94T deserves to be considered as a new and non-obvious composition in its own right as does its products of the process when Inbred PH94T is used as starting material. Applicant points out that PH94T is a unique inbred plant which never before existed until Applicant filed the application and until its deposit of the same.

Therefore, Fullerton et al. does not teach the seed or plant of PH94T, or an F1 seed or plant produced from PH94T. Claim 15, drawn to the PH94T maize plant, has been allowed by the Examiner. Therefore, because Fullerton et al. does not teach PH94T, it can not anticipate nor is it obvious over claims 1-14 and 17-36.

In light of the above, Applicant respectfully requests the Examiner reconsider and withdraw the rejections to claims 1-14 and 17-36 under 35 U.S.C. § 102(b) or 35 U.S.C. § 103(a) as obvious over Fullerton et al., (U.S. Patent 6,169,234).

Request for Information under 37 C.F.R. § 1.105

The Examiner has made a Request for Information under 37 C.F.R. § 1.105. The Examiner states the requested information is "required to make a meaningful and complete search of the prior art". *See* Office Action– Request for Information Under 37 C.F.R. § 1.105, p. 7.

Applicant provides answers to each of the Examiner's interrogatories discussed *infra*.

The Examiner begins by asking firstly, what were the original parental maize lines used to produce maize inbred line PH94T? Please supply information pertaining to the lineage of the original parental lines back to any publicly available varieties. PHME2 and PH1K2. Information pertaining to the lineage of the original parental lines is available within the PVP Application No. 200200192, attached as Appendix 4.

Secondly, what method and steps were used to produce maize inbred line PH94T? Pedigree selection method produced by selfing for 8 generations.

Third, have any of said parental maize lines or progeny therefrom been disclosed or made publicly available?

a. The parental maize line PHME2 has not been previously disclosed or made publicly available. The parental maize line PH1K2 was previously disclosed or made publicly available in PVP Certificate No. 9900376 and U.S. Patent No. 6,124,534.

b. No other progeny of the parental cross PHME2/PH1K2 was previously disclosed or made publicly available by Applicant prior to the earliest priority date.

Fourth, were any other maize lines produced by said method using said original parental maize lines, and if so, have said produced maize lines been publicly available or sold? If so, under what designation/denomination and under what conditions were said other maize lines disclosed or made publicly available? No other maize line using the same F1 cross has been produced by said method using said original parental maize lines at or before the time of filing of the instant application.

In light of the above remarks, Applicant respectfully requests reconsideration and compliance with the interrogatories under the Request for Information under 37 C.F.R. § 1.105.

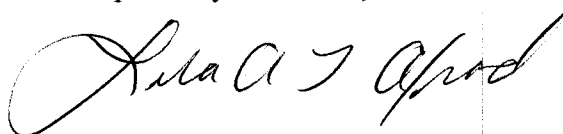
Conclusion

In conclusion, Applicant submits in light of the above amendments and remarks, the claims as amended are in a condition for allowance, and reconsideration is respectfully requested. If it is felt that it would aid in prosecution, the Examiner is invited to contact the undersigned at the number indicated to discuss any outstanding issues.

No other fees or extensions of time are believed to be due in connection with this amendment; however, consider this a request for any extension inadvertently omitted, and charge any additional fees to Deposit Account No. 26-0084.

Reconsideration and allowance is respectfully requested.

Respectfully submitted,

A handwritten signature in black ink, appearing to read "Lila A. T. Akrad". The signature is fluid and cursive, with a large initial "L" and a stylized "A".

Lila A. T. Akrad, Reg. No. 52,550
McKEE, VOORHEES & SEASE, P.L.C.
801 Grand Avenue, Suite 3200
Des Moines, Iowa 50309-2721
Phone No: (515) 288-3667
Fax No: (515) 288-1338
CUSTOMER NO: 27142

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Attorneys of Record

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Marker-assisted backcrossing: a practical example

M. RAGOT¹, M. BIASIOLLI¹, M.F. DELBUT², A. DELL'ORCO¹, L. MALGARINI¹,
 P. THEVENIN¹, J. VERNON², J. VIVANT², R. ZIMMERMANN¹ and G. GAY¹.

¹ Ciba Seeds, CH-4002 Basle, Switzerland.

² Département d'Amélioration des Plantes, INRA-Domaine d'Epoisses,
 F-21110 Genlis, France

Summary

That molecular markers allow fast recovery of recurrent parent genotype in backcross programs is undisputed. Restriction Fragment Length Polymorphisms (RFLP's) were used in maize to introgress by backcross a transgene construct, containing phosphinothricin resistance and insecticidal protein genes, from a transformed parent into an elite inbred line. At each generation plants carrying the transgene construct were selected based on their phosphinothricin resistance, and further characterized with RFLP's. Both maximum recovery of recurrent parent genotype and minimum linkage drag were taken into account for marker-based selection. Embryo rescue was used to shorten generation time. Progress towards recurrent parent genotype was spectacular. Levels of recurrent parent genotype recovery which would normally be observed, in the absence of selection, in the BC₆ generation were obtained at the BC₃ generation, about one year after BC₁ seeds had been planted. Besides the evidence already provided by RFLP's, phenotypic evaluation of the backcross-derived near-isogenic lines will constitute an additional check of the completeness of the conversion.

Introduction

Backcrossing has been a common breeding practice for as long as elite germplasm has been available. It has mainly been used to introgress single Mendelian traits, such as disease resistances or quality factors, into elite germplasm (Allard 1960; Hallauer and Miranda 1981). One of the most attractive attributes of backcrossing is that it allows to perform targeted modifications without disrupting the existing overall genetic balance of the recurrent parent.

However, production of fully converted near isogenic lines through classical backcrossing procedures is a lengthy procedure, if at all possible. Theoretically, a minimum

of seven classical backcross generations are required to recover more than 99% of recurrent parent genotype, assuming no linkage drag. The attractiveness of classical backcross procedures is therefore substantially diminished for crops, such as maize (*Zea mays* L.), where the turn-over of elite cultivars is very fast. In addition, full recovery of recurrent parent genotype is usually not achieved through classical backcrossing, which may result in deleterious agronomic effects. Murray *et al.* (1988) reported about 90% recurrent parent genotype recovery in two BC₁₀-equivalent conversions (A632Ht and A632Rp) of the maize line A632. The conversions had retained respectively 4 and 7 donor fragments in addition to the one carrying the gene of interest.

Reduction in the number of backcross generations needed to obtain fully converted individuals has been shown theoretically, or from simulations, to be achievable through the use of molecular markers (Tanksley *et al.* 1989; Hospital *et al.* 1992; Jarboe *et al.* 1994). Because they provide thorough characterization of the genetic variability at each backcross generation, markers allow to take full advantage of this variability by applying the highest possible selection intensity.

Efficiency of marker-assisted backcrossing was investigated through an experiment aimed at introgressing a single genetic factor (a transgene construct) from a donor into a recipient maize line.

Materials and methods

Plant Material

A hemizygous transgenic maize line of Lancaster origin was used as donor parent to introgress its transgene construct, through repeated backcrossing, into a recipient parent from the Stiff Stalk germplasm group. Both parents are proprietary elite lines. The transgene construct carries both a phosphinothricin resistance gene and synthetic genes encoding the entomotoxic fragment of the CryIA(b) *Bacillus thuringiensis* protein (Kozicel *et al.* 1993). Transformation was achieved through microprojectile bombardment (Kozicel *et al.* 1993) and resulted in a single insertion (*Bt* locus), on chromosome 1 (Figure 1).

Backcross protocol

The F1 progeny of the cross between the donor and the recipient was screened for the presence of the transgene construct by applying Basta, a phosphinothricin-based herbicide, onto each plant. Resistant individuals were then used to generate BC₁ progeny.

For each backcross generation, except the BC₄, individuals were planted in multipots and sprayed with Basta to eliminate those which did not carry the transgene construct. To avoid the stress resulting from treatment with Basta, BC₄ plants carrying the transgene construct were identified using Southern blots probed with the *pat* and *Bt* genes. Resistant plants were transplanted in an open-soil greenhouse and leaf-sampled for molecular marker

analyses. Results of marker analysis were obtained before flowering. A single plant was rescued and transferred onto tissue culture medium, before being averaged, four months.

Molecular marker analysis

Restriction Fragment Length Polymorphism (RFLP) genotypes in all four generations were determined using chemiluminescent techniques. Loci were chosen from among those that provided coverage of the entire genome, contained two loci tightly linked to the gene of interest (Fig. 1), and were present in the BC_{n+1} generation comprised both of tightly linked ones, and additional selected BC_n plant was heterozygous for independent reference population generation.

Selection procedure

At each generation plants of recurrent-parent-genotype and of transgenic-parent-genotype were selected to attempt to integrate both criteria. Missing values were not included. The best ranking one of those for which the BC₃ selection) was available.

Results and discussion

Selection for the gene of interest

The observed segregation was significantly different ($P < 0.05$).

Recurrent parent genotype

Statistics for the genotype were performed taking the whole genome of backcross-derived plant thereof.

recover more than 99% of recurrent
tractiveness of classical backcross
ops, such as maize (*Zea mays* L.).
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al *et al.* 1992; Jarboc *et al.* 1994).
genetic variability at each backcross
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investigated through an experiment
the construct) from a donor into a

origin was used as donor parent to
backcrossing, into a recipient parent
are proprietary elite lines. The
distance gene and synthetic genes
Bt *thuringiensis* protein (Koziel *et*
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chromosome 1 (Figure 1).

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phosphinothricin-based herbicide,
generate BC₁ progeny.

Individuals were planted in multipots
carry the transgene construct. To
BC₁ plants carrying the transgene
with the *pat* and *Bt* genes. Resistant
leaf-sampled for molecular marker

analyses. Results of marker analyses were made available at the latest two weeks after
flowering. A single plant was selected, of which all backcross-derived embryos were
rescued and transferred onto tissue culture medium. Plantlets that developed from these
embryos first underwent a greenhouse acclimation phase, while still growing on tissue
culture medium, before being transplanted into multipots. Backcross cycles lasted, on
average, four months.

Molecular marker analyses

Restriction Fragment Length Polymorphisms (RFLP's) were used to establish
genotypes in all four generations. RFLP detection involved either radioactive or
chemiluminescent techniques. For the BC₁ generation, 61 marker-enzyme combinations
were chosen from among those revealing polymorphism between donor and recipient. They
provided coverage of the entire genome, defining intervals of about 25 cM in size, and
contained two loci tightly linked to the *Bt* locus, CG320 and CG415, respectively 5 and 16
recombination units away (Figure 1). For subsequent generations, markers analyzed in the
BC_{n+1} generation comprised both those for which the selected BC_n plant was heterozygous,
or tightly linked ones, and additional ones located in chromosomal segments for which the
selected BC_n plant was heterozygous (Table 1). Marker map positions were obtained from
independent reference populations and confirmed by analysis of segregation in the BC₁
generation.

Selection procedure

At each generation plants were ranked based both on the percentage of homozygous
recurrent-parent-genotype and on the extent of linkage drag around the *Bt* locus, in an
attempt to integrate both criteria. Plants for which two or more adjacent markers had
missing values were not included in the analyses. Success or failure of the pollinations also
contributed to the selection procedure. One single plant was selected at each generation: the
best ranking one of those for which a backcross progeny of size 100 or more (50 or more
for the BC₃ selection) was available.

Results and discussion

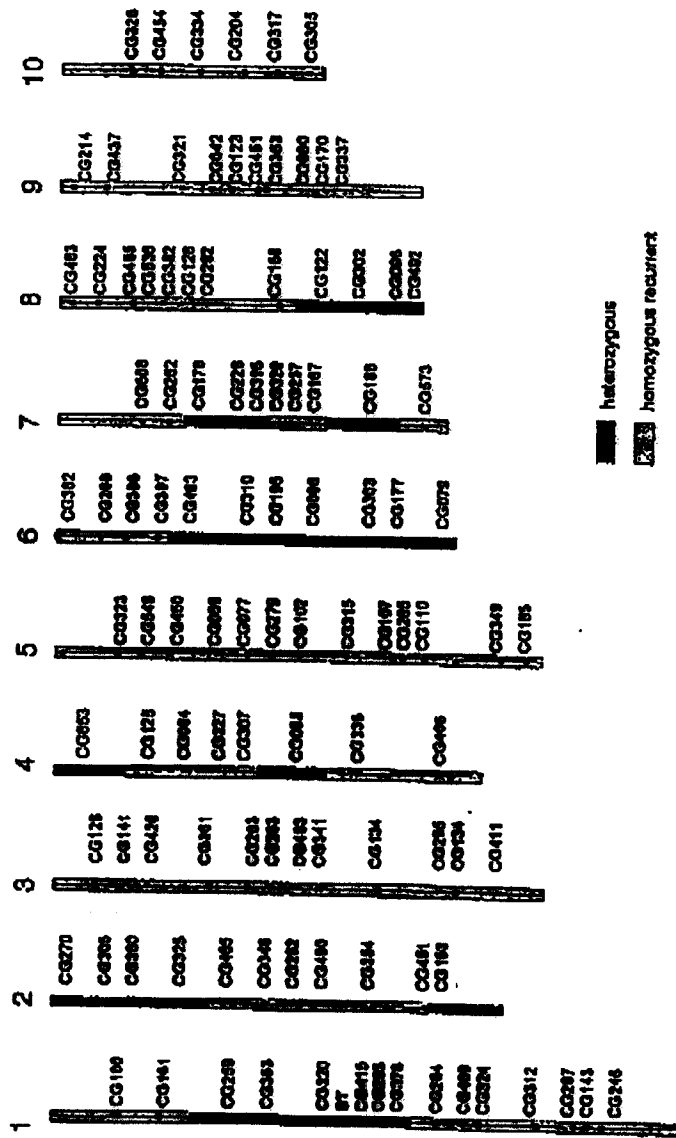
Selection for the gene of interest

The observed segregation ratios for phosphinothricin resistance (Table 1) were not
significantly different ($P < 0.05$) from the expected 1:1, as shown by Chi-square tests.

Recurrent parent genotype recovery

Statistics for the genotyped plants are summarized in Table 1. Calculations were
performed taking the whole genome into account, including the *Bt* locus. The "perfect"
backcross-derived plant therefore counts one heterozygous chromosome segment, that

SELECTED BC1

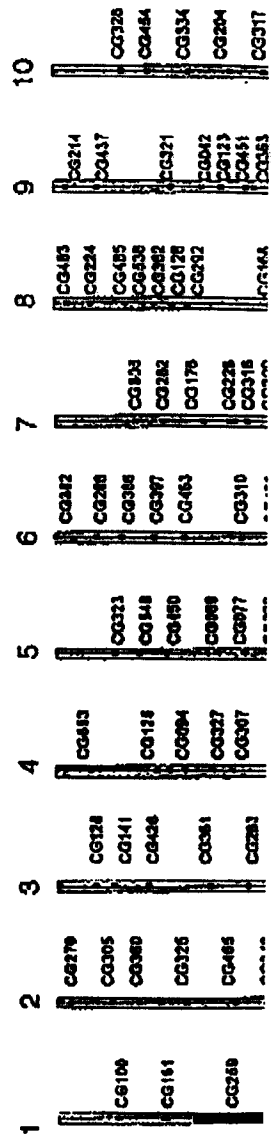


heterozygous

homozygous recurrent

Figure 1-a: Genetic maps of the backcross-derived individuals selected in the first four generations of a marker-assisted backcross program. The locus to be introgressed (Bt) is located on chromosome 1.

SELECTED BC2

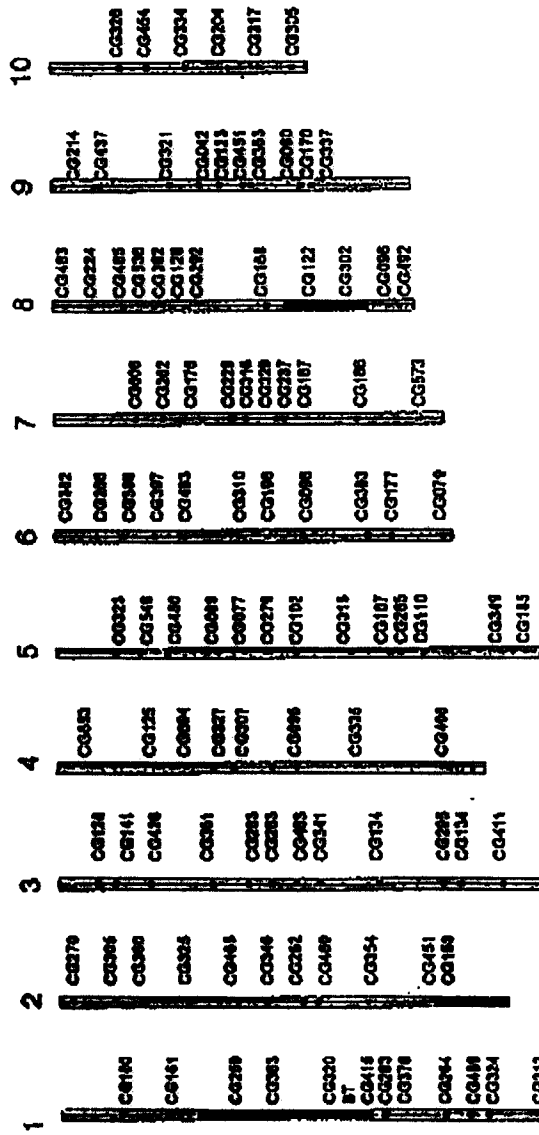




■ heterozygous
 ■ homozygous recurrent

Figure 1-a: Genetic maps of the backcross-derived individuals selected in the first four generations of a marker-assisted backcross program. The locus to be introgressed (*Bt*) is located on chromosome 1.

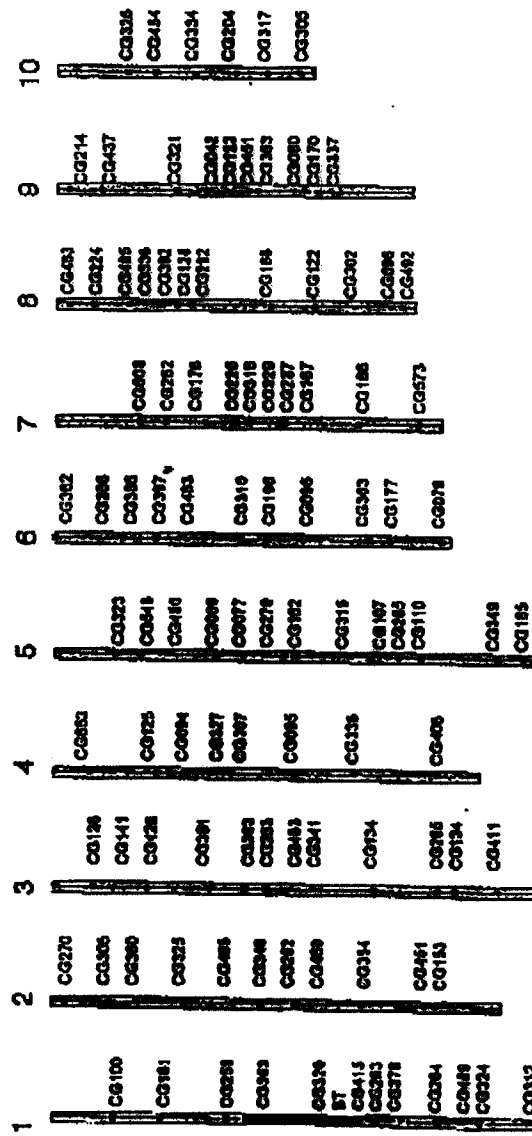
SELECTED BC2



■ heterozygous
 ■ homozygous recurrent

Figure 1-b: Genetic maps of the backcross-derived individuals selected in the first four generations of a marker-assisted backcross program. The locus to be introgressed (*Bt*) is located on chromosome 1.

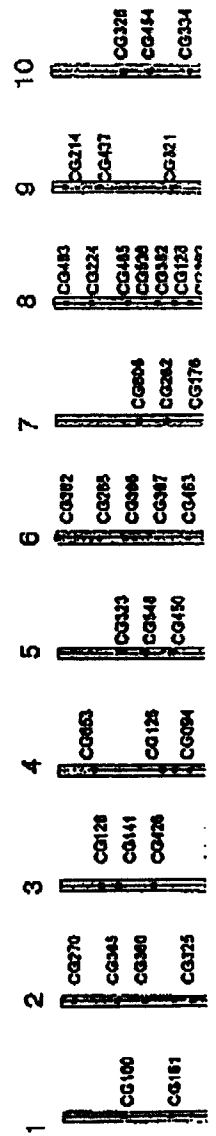
SELECTED BC3



heterozygous
homozygous recurrent

Figure 1-c: Genetic maps of the backcross-derived individual selected in the first four generations of a marker-assisted backcross program. The focus to be introgressed (*Bt*) is located on chromosome 1.

SELECTED BC4



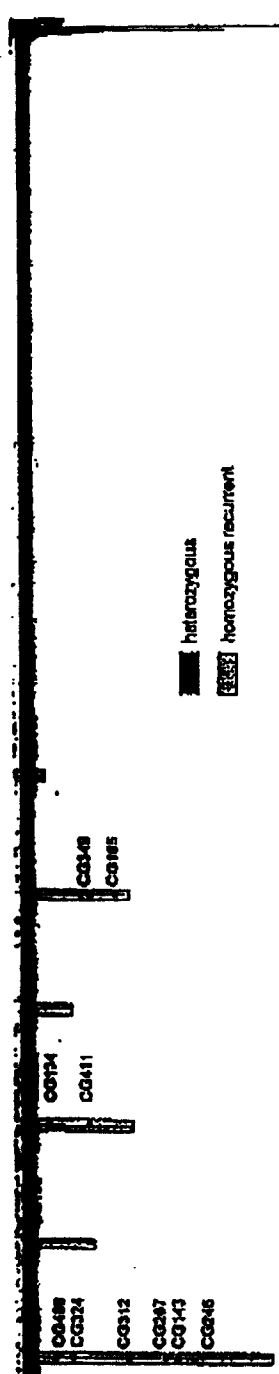
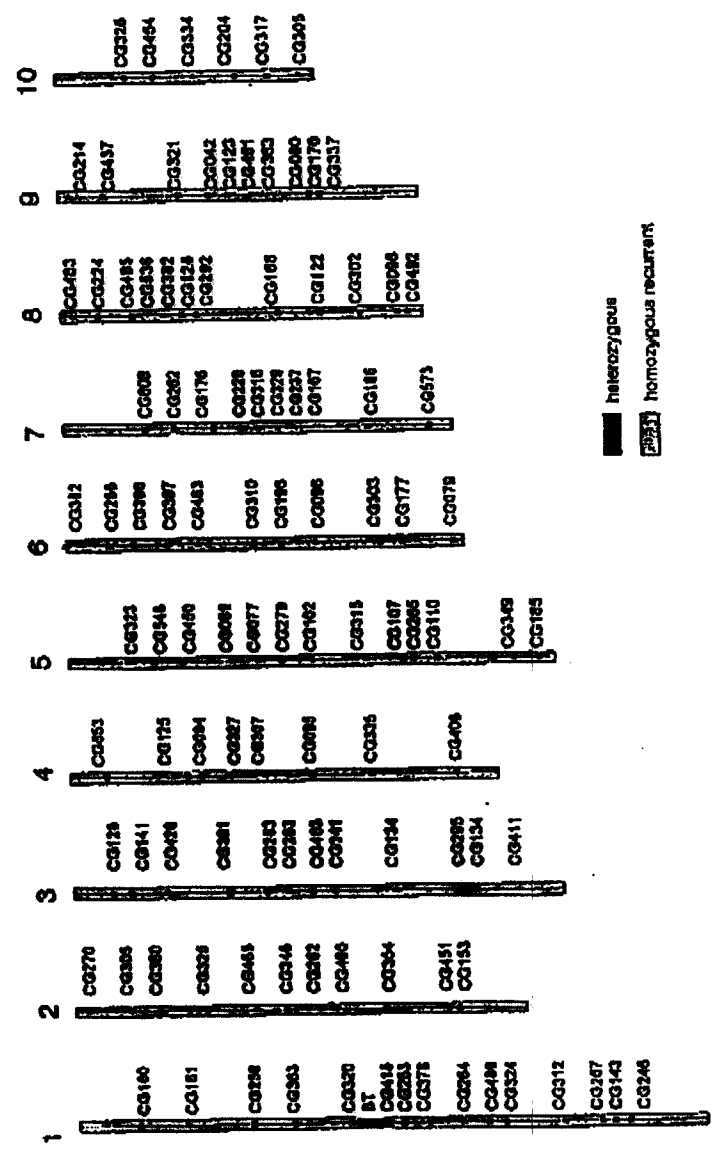


Figure 1-c: Genetic maps of the backcross-derived individuals selected in the first four generations of a marker-assisted backcross program. The locus to be introgressed (*Bt*) is located on chromosome 1.

SELECTED BC4



■ heterozygous
□ homozygous recurrent

Figure 1-d: Genetic maps of the backcross-derived individuals selected in the first four generations of a marker-assisted backcross program. The locus to be introgressed (*Bt*) is located on chromosome 1.

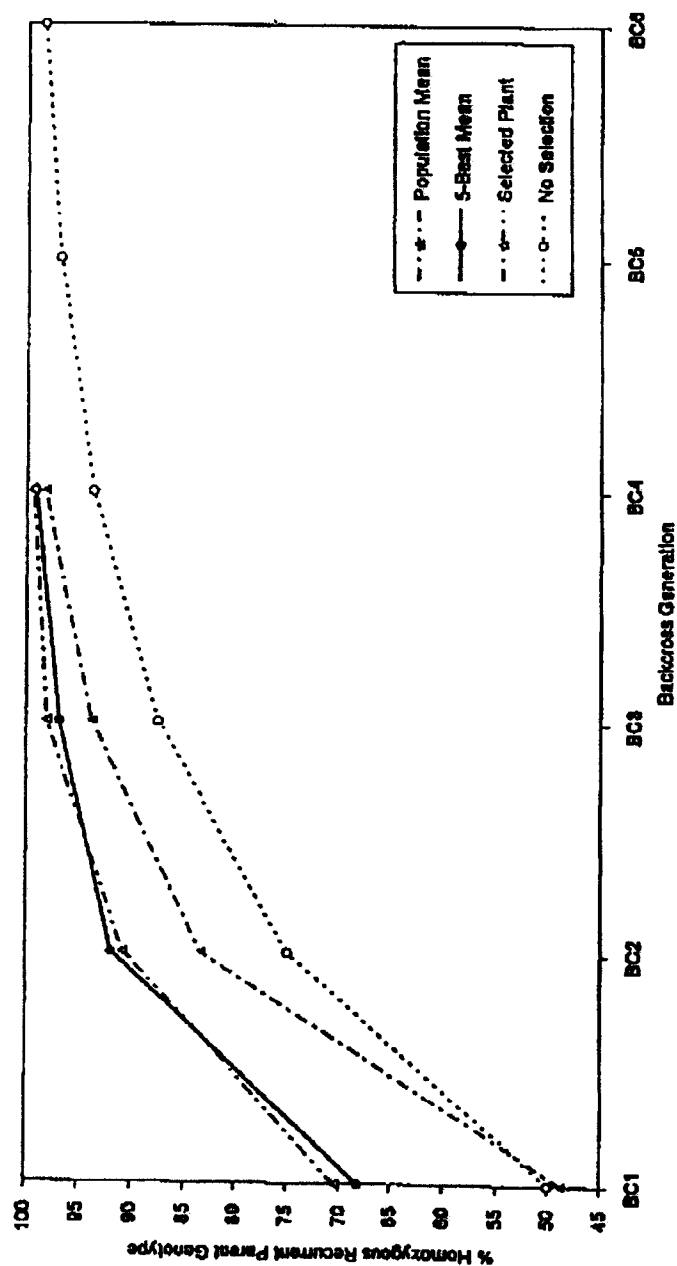


Figure 2: Recovery of recurrent parent genotype through backcrossing, with or without marker-assisted selection

Table 1: Proportion and characteristics of plants carrying the genes of interest, in the first four generations of a marker-assisted backcross program.

generation	% chloramphenicol	RFLP genotyping	nb plants	% homozygous recurrent	nb heterozygous
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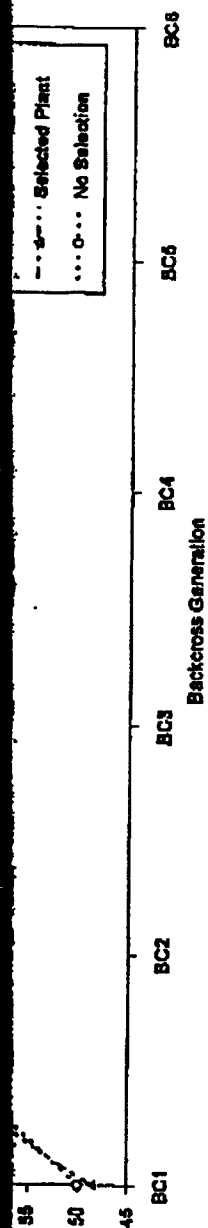


Figure 2: Recovery of recurrent parent genotype through backcrossing, with or without marker-assisted selection

Table 1: Proportion and characteristics of plants carrying the genes of interest, in the first four generations of a marker-assisted backcross program.

generation	% phosphinothricin resistant plants	RFLP genotyping			nb plants analyzed *	% homozygous recurrent parent genotype				nb heterozygous chromosome segments ***			
		nb plants	nb loci	nb datapoints		mean	std dev	5-best mean **	selected plant	mean	std dev	5-best mean **	selected plant
BC1	40.68	90	61	9660	57	66.72	10.36	66.31	70.45	11.01	2.17	7.75	0
BC2	44.95	61	23	1343	50	83.42	8.84	81.98	80.44	8.05	1.84	3.20	9
BC3	46.32	72	10	720	71	83.83	1.85	86.82	83.03	2.20	0.71	1.60	1
BC4	.	26	3	78	26	98.23	0.49	98.09	98.38	1.00	0.90	1.00	1

* Plants for which two or more adjacent markers had missing values were not included in the analyses

** Mean value of the five individuals having the five highest percentages of homozygous recurrent parent genotype.

*** Including the segment carrying the transgene construct.

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fragment length polymorphisms: what

homozygous recurrent-parent-genotype. The relative length of the chromosome between the two flanking markers chosen.

The parent-genotype of the BC₁ generation was explained by linkage drag around the *Bt* locus based only on plants selected for the first generation the mean percentage of *Bt* was higher than what would have been expected (Figure 2).

The parent-genotype of the selected plant (Table 1) were always very similar to the parent value (Figure 2). The percentage of *Bt* in the selected plant was found only once, in the five largest values. This corresponded to one with the maximum percentage of *Bt* and had been selected because it displayed a *Bt* phenotype (Figure 1).

The parent-genotype of the selected BC₁ plant of the selected BC₂ was larger than that of the parent, but smaller than that of an unselected plant of the "perfect" backcross-derived population. The rates of recurrent parent genotype in the backcrosses. Jarboe *et al.* (1994) who used backcross generations and 80 markers to estimate the *Bt* locus.

The segments decreased from one backcross generation were not necessarily those which were selected (Table 1). However, with the first backcross which contained only one *Bt* locus.

The relative to the length of chromosome was 4% for the selected BC₁ individual, between 2.0 and 24.0% for the selected BC₂ and 14.5 cM for the selected BC₄.

The two values given for each generation are extreme values of linkage drag, which correspond to extreme positions of the crossing-overs in the marker-defined intervals flanking the transgene construct locus. Therefore the true linkage drag value of the selected BC₄ is likely to be less than 1.3% of the genome. Although this maximum value may appear to be somewhat high, reflecting the limited selection pressure put here on linkage drag, it is much lower than what would be expected from classical backcross programs (Stam and Zeven 1981; Tanksley *et al.* 1989). Practically, in a study of *Tm-2* conversions of tomato cultivars obtained by a large number of classical backcross cycles, Young and Tanksley (1989) found that the sizes of the introgressed fragments ranged between 4 and 51 cM.

Conclusion

These results clearly demonstrate that molecular markers provide important time and quality advantages over classical procedures for the production of near-isogenic lines through backcrossing. Only four backcross generations were necessary to recover, in less than a year and a half from planting of the BC₁'s, individuals which appeared to be genotypically fully converted. Nevertheless, it is likely that recovery of recurrent parent genotype could proceed even faster than in the experiment described herein, should the appropriate protocol and resources (population size, number and position of markers) be allocated.

Comparison of BC₄-derived lines with the recurrent parent for both morphological markers and agronomic performance (including hybrid performance) will be performed in order to confirm the completeness of the conversion.

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C

Marker-assisted Selection in Backcross Breeding

S.J. Openshaw

Pioneer Hi-Bred Intl. Inc., P.O. Box 1004, Johnston, IA 50131

S.G. Jarboe¹

CIMMYT, Lisboa 27, Apdo. Postal 6-641, 06600 Mexico, D.F., Mexico

W.D. Beavis

Pioneer Hi-Bred Intl. Inc., P.O. Box 1004, Johnston, IA 50131

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Abstract. The backcross breeding procedure has been used widely to transfer simply inherited traits into elite genotypes. Genetic markers can increase the effectiveness of backcrossing by 1) increasing the probability of obtaining a suitable conversion, and 2) decreasing the time required to achieve an acceptable recovery. Simulation and field results indicated that, for a genome consisting of ten 200-cM chromosomes, basing selection on 40 or 80 markers in 50 BC individuals that carry the allele being transferred can reduce the number of backcross generations needed from about seven to three.

The backcross breeding procedure has been used widely to transfer simply inherited traits into elite genotypes. Usually, the trait being transferred is controlled by a single gene, but highly heritable traits that are more complexly inherited have also been transferred successfully by backcrossing; for example, maturity in maize (Rinks and Sentz, 1961; Shaver, 1976). Today, backcrossing is being used to transfer genes introduced by such techniques as transformation or mutation into appropriate germplasm.

Several plant breeding textbooks give good descriptions of the backcross procedure (Allard, 1960; Febr, 1987). A donor parent (DP) carrying a trait of interest is crossed to the recurrent parent (RP), an elite line that is lacking the trait. The F₁ is crossed back to the RP to produce the BC₁ generation. In the BC₁ and subsequent backcross generations, selected individuals carrying the gene being transferred are backcrossed to the RP. The expected proportion of DP genome is reduced by half with each generation of backcrossing. Ignoring effects of linkage to the selected DP allele being transferred, the percentage recurrent parent (%RP) genome expected in each backcross generation is calculated as:

$$\%RP = 100 [1 - (0.5)^n]$$

where n is the number of backcrosses.

Backcrossing of selected plants to the RP can be repeated each cycle until a line is obtained that is essentially a version of the RP that includes the introgressed allele. After six backcrosses, the expected recovery is >99% (Table 1).

Until recently, discussions of the recovery of the RP genome during backcrossing have emphasized the expected values for

%RP shown in Table 1, and have largely ignored the genetic variation for %RP that exists around the expected mean. With the development of genetic markers capable of providing good genome coverage, there has been interest in taking advantage of that variation to increase the efficiency of backcrossing.

Selection for RP marker alleles can increase greatly the effectiveness of backcross programs by allowing the breeder to 1) select backcross plants that have a higher proportion of RP genome, and 2) select backcross individuals that are better conversions near a mapped donor allele being transferred (i.e., select for less linkage drag). Expressed in practical terms, using genetic markers to assist backcrossing can 1) increase the probability of obtaining a suitable conversion, and 2) decrease the time required to achieve an acceptable recovery.

Issues to consider when planning a marker-assisted backcross program include 1) the time advantage of using markers to assist backcrossing, 2) the number of markers needed, and 3) the number of genotypes to evaluate. In this report, we use results from previous literature, computer simulation, and empirical studies to provide some guidelines.

Table 1. Expected recovery of recurrent parent (RP) genome during backcrossing, assuming no linkage to the gene being transferred.

Generation	%RP
F ₁	50.0000
BC ₁	75.0000
BC ₂	87.5000
BC ₃	93.7500
BC ₄	96.8750
BC ₅	98.4375
BC ₆	99.2188
BC ₇	99.6094

¹Formerly with Purdue University, West Lafayette, Ind.

Materials and methods

The maize genome was the model for the simulation. The simulated genome contained ten 200-cM chromosomes. Simulation of crossing over was based on a Poisson distribution with a mean of 2.0 ($\lambda = 2$) (Hanson, 1959), which, on average, generated one cross over for every 100-cM length. The simulations reported here assume no interference. Codominant genetic markers were evenly distributed in the genome and sites of the donor gene were randomly assigned to genome locations.

Simulations were conducted with the following parameters:

Number of progeny: 100 or 500.

Backcross generations: BC_1 , BC_2 , and BC_3 .

Number of markers: 20, 40, 80, or 100.

Number selected to form the next BC generation: 1 or 5.

Selection was based on 1) presence of the donor allele and 2) high %RP. %RP was calculated as the average of the (one or five) selected individuals. Values presented are the mean of 50 simulations.

Results

In the computer simulation study, all methods modeled greatly increased the speed of recovering the RP genome compared to the expected recovery with no marker-assisted selection (compare Tables 1 and 2). At least 80 markers were required to recover 99% of the RP genome in just three BC generations (Table 2). Use of at least 80 markers and 500 progeny allowed recovery of 98% RP in just two BC generations. Response to selection was diminished only slightly by spreading the effort over five selections. Using markers, the number of backcross generations needed to convert an inbred is

reduced from about seven to three.

By the BC_3 generation, there appears to be no practical advantage to using 500 vs. 100 individuals. If the presence of the donor trait in the backcross individuals can be ascertained before markers are genotyped, then only half the number of individuals indicated in the tables will need to be analyzed.

When a small number of markers are used, they quickly become non-informative; i.e., selection causes the marker loci to become fixed for the RP type before the rest of the genome is fully converted (Table 3; Hospital et al., 1992). This situation was most prominent in the larger populations, where a higher selection intensity placed more selection pressure upon the marker loci. Accordingly, it is of interest to consider how closely the estimation of %RP based on markers reflects the actual genome composition. The combination of estimation of %RP based on fewer markers and subsequent selection tends to bias the estimates upward (compare Tables 2 and 3).

The results from the simulation compare well with real field data. In a typical example, 50 BC_2 plants carrying the gene being transferred were genotyped at 83 polymorphic RFLP loci (note that this corresponds to a population size of 100 unselected plants in Tables 2 and 3). The five best BC_2 recoveries had estimated %RP values of 85.9%, 82.7%, 82.0%, 81.4%, and 81.2%. After evaluating 10 BC_3 plants from each selected BC_2 , the best BC_3 recovery had an estimated %RP of 94.6%.

Discussion

The simulations (Table 2; Hospital et al., 1992) and our experience indicate that four markers per 200-cM chromosome is adequate to greatly increase the effectiveness of selection in the BC_2 . However, using only four markers per 200 cM will likely make it very difficult to map the location of the gene of interest. Adequate summarization of the data is an important

Table 2. Percent recurrent parent genome during marker-assisted backcrossing.

Generation	100 Progeny				500 Progeny			
	No. markers				No. markers			
	20	40	80	100	20	40	80	100
One selected								
BC_1	84.5	84.5	84.2	88.0	89.9	90.7	90.2	90.5
BC_2	95.0	95.2	95.4	97.2	96.5	97.7	98.5	98.6
BC_3	97.4	97.6	98.0	99.2	97.7	98.3	99.4	99.5
Five selected								
BC_1	82.9	85.1	84.9	84.7	87.7	88.1	88.9	88.9
BC_2	93.7	95.0	95.8	95.7	95.5	96.8	97.8	97.9
BC_3	97.1	98.3	98.8	98.9	97.3	98.5	99.3	99.3

Table 3. Estimates of percent recurrent parent genome, based on marker loci.

Generation	100 Progeny				500 Progeny			
	No. markers				No. markers			
	20	40	80	100	20	40	80	100
One selected								
BC_1	98.7	97.8	95.6	97.2	100.0	99.1	98.6	98.0
BC_2	100.0	99.8	99.3	99.3	100.0	100.0	99.9	98.2
Five selected								
BC_1	96.4	96.5	96.2	95.8	100.0	98.5	98.3	98.2
BC_2	99.9	99.8	99.3	99.1	100.0	100.0	99.9	99.8

part of a marker-assisted backcross program. Ideally, the markers used can supply data that can be represented as alleles of loci with known map position. Estimation of %RP, mapping the position of the locus of interest, and graphical display of the results (Young and Tanksley, 1989) are all useful in understanding and controlling the specific backcross experiment being conducted.

It appears that, with the use of genetic markers, the portion of the RP genome that is not linked to the allele being transferred can be recovered quickly and with confidence. The recovery of RP will be slower on the chromosome carrying the gene of interest. A considerable amount of linkage drag is expected to accompany selection for the DP allele in a backcross program. For a locus located in the middle of a 200-cM chromosome, the length of the DP chromosome segment accompanying selection is expected to be 126, 63, and 28 cM in the BC₁, BC₂, and BC₃ generations, respectively (Hanson, 1959; Naveira and Barbadilla, 1992). Our observations support the recommendation of Hospital et al. (1992) that preference be given to the selection for recombinants proximal to the allele of interest, but that selection for recovery of the RP elsewhere in the genome also be considered. This two-stage selection can probably be done quite effectively ad hoc by the breeder once the data is adequately summarized; however, Hospital et al.

suggest ways to incorporate the two criteria into a selection index such that each component of selection is assured appropriate weighting.

Use of genetic markers can greatly increase the effectiveness of backcrossing, and they should be used in any serious backcrossing program if resources are available to the breeder.

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J. S. C. Smith · E. C. L. Chin · H. Shu
O. S. Smith · S. J. Wall · M. L. Senior
S. E. Mitchell · S. Kresovich · J. Ziegler

An evaluation of the utility of SSR loci as molecular markers in maize (*Zea mays* L.): comparisons with data from RFLPs and pedigree

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Abstract The utility of 131 simple sequence repeat (SSR) loci to characterize and identify maize inbred lines, validate pedigree, and show associations among inbred lines was evaluated using a set of 58 inbred lines and four hybrids. Thirteen sets of inbred parent-progeny triplet pedigrees together with four hybrids and their parental lines were used to quantify incidences of scoring that departed from expectations based upon simple Mendelian inheritance. Results were compared to those obtained using 80 restriction fragment length polymorphism (RFLP) probes. Over all inbred triplets, 2.2% of SSRs and 3.6% of RFLP loci resulted in profiles that were scored as having segregated in a non-Mendelian fashion. Polymorphic index content (PIC, a measure of discrimination ability) values ranged from 0.06 to 0.91 for SSRs and from 0.10 to 0.84 for RFLPs. Mean values for PIC for SSRs and RFLPs were similar, approximately 0.62. However, PIC values for nine SSRs exceeded the maximum PIC for RFLPs. Di-repeats gave the highest mean PIC scores for SSRs but this class of repeats can result in "stutter" bands that complicate accurate genotyping. Associations among inbreds were similar for SSR and RFLP data,

closely approximating expectations from known pedigrees. SSR technology presents the potential advantages of reliability, reproducibility, discrimination, standardization and cost effectiveness over RFLPs. SSR profiles can be readily interpreted in terms of alleles at mapped loci across a broad range of maize germ plasm. Consequently, SSRs represent the optimum approach for the identification and pedigree validation of maize genotypes compared to other currently available methods.

Key words Simple sequence repeat · Microsatellite · SSRs · Maize · Variety identification

Introduction

Microsatellites, or simple sequence repeats (SSRs) are short nucleotide sequences, usually from 2 to 3 bases(b) in length that are repeated in tandem arrays. Amplifiable polymorphisms are revealed because of differences in the numbers of tandem repeats that lie between sequences that are otherwise conserved for each locus. Microsatellite loci have proven to be highly polymorphic and useful as genetic markers in many plant species including *Arabidopsis* (Depeiges et al. 1995), bur oak (Dow et al. 1995), maize (Senior and Heun 1993), seashore paspalum (Liu et al. 1995), rapeseed (Kresovich et al. 1995; Charters et al. 1996), soybean (Akkaya et al. 1992, 1995; Rongwen et al. 1995), sugar beet (Mörchen et al. 1996), sweet potato (Jarret and Bowen 1994) and wheat (Plaschke et al. 1995; Roder et al. 1995).

In this paper, we report the usefulness of SSRs as genetic markers to discriminate between, and to show associations among, inbred lines of maize using a greater number of loci and a broader diversity of maize germ plasm than has been reported previously (Senior and Heun 1993).

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J. S. C. Smith (✉) · E. C. L. Chin · H. Shu
O. S. Smith · S. J. Wall
Pioneer Hi-Bred International, Inc., Trait and Technology
Development, Research and Product Development, 7300 NW
62nd Avenue-P.O. Box 1004, Johnston, IA 50131-1004, USA

M. L. Senior
Department of Crop Science, North Carolina State University,
Raleigh, NC 27695, USA

S. E. Mitchell · S. Kresovich
USDA-ARS, Plant Genetic Resources Conservation Unit,
1109 Experiment Street, Griffin, GA 30223-1197, USA

J. Ziegler
Perkin Elmer-Applied Biosystems Division, 850 Lincoln Center
Drive, Foster City, CA 94404, USA

Table 1 List and pedigree background of inbred lines used in the present SSR and RFLP profiling study

A632	Pedigree background ^a
A632	BSSS ^b C0 (94%), Minnesota 13 ^c (6%)
B73	BSSS ^b (100%)
Mo17	Lancaster Sure Crop ^c (50%), Krug ^c (50%)
PH207	Iodent ^c (59%), Long Ear ^c (20%), Minnesota 13 ^c (11%), Troyer Reid ^c (5%)
B64	BSSS ^b C0 (87.5%), Maiz Amargo ^c (12.5%)
PH595	Midland Yellow Dent ^c (25%), Southern U.S. Landrace Synthetic (19%), Funks G4949 (12.5%), Illinois Long Ear ^c (12.5%), Illinois Two Ear (12.5%)
PH642	BSSS ^b C0 (87.5%), Iodent ^c (9%)
PH814	Lancaster Low Breakage (25%), Southern U.S. Landrace Synthetic (19%), Osterland Yellow Dent ^c (16%), Funks G4949 (13%), Midland Yellow Dent ^c (6%), Tuson R ^c (6%), Brookings 86 ^c (5%)
PH848	Minnesota 13 ^c (12.5%), Osterland Yellow Dent ^c (12.5%), SRS303 ^c (12.5%), Iodent ^c (12%), Reid Yellow Dent ^c (12%), Lancaster Sure Crop ^c (6%), Longfellow Flint ^c (6%), MHW ^d (6%)
PHB09	BSSS ^b C0 (62.5%), Minnesota 13 ^c (25%)
PHB46	BSSS ^b C0 (50%), Alberta Flint ^c (25%), Osterland Yellow Dent ^c (25%)
PHB47	BSSS ^b C0 (87.5%), Brookings 86 ^c (12.5%)
PHB76	Smith TC ^c (25%), Midland Yellow Dent ^c (12.5%), NW Dent ^c (12.5%), Southern U.S. Landrace Synthetic (9%), Minnesota 13 ^c (8%), Funks G4949 (6%), Illinois Long Ear (6%), Illinois Two Ear ^c (6%), Osterland Yellow Dent ^c (6%)
PHB89	Coker 616 (25%), Lancaster Sure Crop ^c (12.5%), Midland Yellow Dent ^c (12.5%), Southern U.S. Landrace Synthetic (9%), Minnesota 13 ^c (8%), Funks G4949 (6%), Funks Yellow Dent ^c (6%), Illinois Long Ear ^c (6%), Illinois Two Ear (6%), Iodent ^c (18%), Southern U.S. Landrace Synthetic (9%), Minnesota 13 ^c (9%), Osterland Yellow Dent ^c (6%), Midland Yellow Dent ^c (6%), Long Ear (6%), Funks G4949 (6%), Lancaster Low Breakage (5%)
PHBG4	Iodent ^c (27%), Minnesota 13 ^c (11%), Long Ear (9%), Coker 616 (8%), Midland Yellow Dent ^c (6%), Lancaster Sure Crop ^c (6%), Southern U.S. Landrace Synthetic (6%)
PHG12	BSSS ^b C0 (37.5%), Lancaster Low Breakage (25%), M3204 ^d (25%)
PHG29	Iodent ^c (59%), Long Ear (20%), Minnesota 13 ^c (13%), Troyer Reid ^c (5%)
PHG31	Iodent ^c (44%), Long Ear (15%), Minnesota 13 ^c (11%), Midland Yellow Dent ^c (6%), Southern U.S. Landrace Synthetic (5%)
PHG35	Iodent ^c (29%), Midland Yellow Dent ^c (13%), Minnesota 13 ^c (11%), Southern U.S. Landrace Synthetic (9%), Long Ear (9%), Funks G4949 (6%), Illinois Long Ear (6%), Illinois Two Ear (6%)
PHG39	BSSS ^b C0 (69%), Maiz Amargo ^c (25%)
PHG42	Iodent ^c (30%), Lancaster Low Breakage (10%), Southern U.S. Landrace Synthetic (9%), Osterland Yellow Dent ^c (9%), Minnesota 13 ^c (7%), Funks G4949 (6%)
PHG45	Iodent ^c (59%), Long Ear (20%), Minnesota 13 ^c (13%), Troyer Reid ^c (5%)
PHG50	Iodent ^c (35%), Long Ear (12%), Minnesota 13 ^c (12%), Osterland Yellow Dent ^c (7%), SRS 303 ^c (6%), Reid ^c (6%)
PHG53	BSSS ^b C0 (91%), Maiz Amargo ^c (6%)
PHG55	PROCOMP ^b (50%), Minnesota 13 ^c (6%), Osterland Yellow Dent ^c (6%), SRS 303 ^c (6%), Iodent ^c (6%), Reid ^c (6%)
PHG69	BSSS ^b (50%), BSSS ^b (50%) BSSS ^b C0 (25%), Alberta Flint (13%), Osterland Yellow Dent ^c (13%)
PHG71	BSSS ^b C0 (47%), Iodent ^c (30%), Long Ear (10%), Minnesota 13 ^c (9%)
PHG74	BSSS ^b C0 (89%), Minnesota 13 ^c (5%)
PHG80	Dockendorf 101 ^c (50%), BSSS ^b C0 (38%)
PHG81	BSSS ^b (50%), Iodent ^c (30%), Long Ear (10%), Minnesota 13 ^c (6%)
PHG83	Iodent ^c (30%), Lancaster Low Breakage (13%), Long Ear (10%), Southern U.S. Landrace Synthetic (9%), Osterland Yellow Dent ^c (9%), Minnesota 13 ^c (7%), Funks G 4949 (6%)
PHG84	Midland Yellow Dent ^c (13%), Southern U.S. Landrace Synthetic (9%), Minnesota 13 ^c (8%), Funks G4949 (6%), Illinois Low Ear (6%), Illinois Two Ear (6%), Osterland Yellow Dent ^c (6%), SRS 303 ^c (6%), Iodent ^c (6%), Reid ^c (6%)
PHG86	BSSS ^b (50%), BSSS ^b C0 (44%), Maiz Amargo ^c (6%)
PHJ76	BSSS ^b (50%), BSSS ^b C0 (38%)
PHK29	BSSS ^b C0 (63%), BSSS ^b (25%), Brookings 86 ^c (6%)
PHK42	Iodent ^c (59%), Long Ear (20%), Minnesota 13 ^c (13%), Troyer Reid ^c (5%)
PHMK0	BSSS ^b C0 (38%), Southern U.S. Landrace Synthetic (21%), BSSS ^b (13%), Dockendorf 101 ^c (13%)
PHMM9	BSSS ^b C0 (53%), Dockendorf 101 ^c (25%), Maiz Amargo ^c (13%)
PHN46	Southern U.S. Landrace Synthetic (12%), Iodent ^c (10%), Lancaster Low Breakage (9%), Osterland Yellow Dent ^c (9%), Funks G4949 (8%), Minnesota 13 ^c (6%), Midland Yellow Dent ^c (6%)
PIIN65	BSSS ^b (50%), Minnesota 13 ^c (6%), Osterland Yellow Dent ^c (6%), SRS 303 ^c (6%), Iodent ^c (6%), Reid ^c (6%)
PIIP38	BSSS ^b C0 (66%), Maiz Amargo ^c (13%), BSSS ^b (13%)
PHP85	BSSS ^b C0 (48%), BSSS ^b (38%), Maiz Amargo ^c (6%)
PHPE5	Iodent ^c (22%), Southern U.S. Landrace Synthetic (9%), Midland Yellow Dent ^c (9%), Minnesota 13 ^c (8%), Long Ear (8%), Coker 616 (6%), Funks G4949 (6%), Illinois Long Ear (5%), Illinois Two Ear (5%)
PHR03	Iodent ^c (25%), Minnesota 13 ^c (11%), Long Ear (8%), Southern U.S. Landrace Synthetic (6%), Midland Yellow Dent ^c (6%), Lancaster Sure Crop ^c (6%)
PHR63	Iodent ^c (29%), Coker 616 (13%), Minnesota 13 ^c (10%), Long Ear (10%), Lancaster Sure Crop ^c (6%), Midland Yellow Dent ^c (6%), Southern U.S. Landrace Synthetic (5%)
PHR92	BSSS ^b C0 (69%), Maiz Amargo ^c (25%)
PHT11	BSSS ^b C0 (47%), BSSS ^b (25%), Maiz Amargo ^c (13%), Alberta Flint (6%), Osterland Yellow Dent ^c (6%)
PHU55	BSSS ^b C0 (69%), Maiz Amargo ^c (25%)
PHV25	Iodent ^c (30%), Midland Yellow Dent ^c (13%), Long Ear (10%), Southern U.S. Landrace Synthetic (9%), Minnesota 13 ^c (7%), Funks G4949 (6%), Illinois Long Ear (6%), Illinois Two ear (6%)

Table 1 Continued

A632	Pedigree background ^a
PHV35	BSSS ^b (50%), BSSS ^b C0 (34%), Maiz Amargo ^c (13%)
PHV78	Iodent ^d (15%), Southern U.S. Landrace Synthetic (14%), Midland Yellow Dent ^e (13%), Funks G4949 (9%), Illinois Long Ear (6%), Illinois Twin Ear (6%), Lancaster Low Breakage (6%), Long Ear (5%), Minnesota 13 ^f (5%), Tuson B ^g (5%)
PHV94	BSSS ^b C0 (53%), Dockendorf 101 ^h (25%), Maiz Amargo ^c (13%)
PHW52	BSSS ^b (50%), BSSS ^b C0 (34%), Maiz Amargo ^c (13%)
PHW53	Iodent ^d (21%), Osterland Yellow Dent ^e (11%), Minnesota 13 ^f (10%), Long Ear (7%), Lancaster Low Breakage (6%), SRS 303 ⁱ (6%), Reid ^j (6%), Southern U.S. Landrace Synthetic (5%)
PHWK9	Maiz Amargo ^c (50%), BSSS ^b C0 (50%)
PHZ38	BSSS ^b (50%), BSSS ^b C0 (41%)
PHZ51	Osterland Yellow Dent ^e (14%), Lancaster Low Breakage (13%), Southern U.S. Landrace Synthetic (9%), Minnesota 13 ^f (8%), Funks G4949 (6%), SRS 303 ⁱ (6%), Iodent ^d (6%), Reid ^j (6%)

^a Contributions of 5% or greater by pedigree are provided

^b Iowa Stiff Stalk Synthetic

^c Open-pollinated variety

^d Derived from Tuson, an open-pollinated variety from the West Indies

^e Population derived from Minnesota 13 open-pollinated variety

^f Stiff Root and Stalk or Stalk Rot Synthetic selection from Krug

^g Dawes open-pollinated variety from Nebraska most likely from Reid obtained from Mount Haleb, Wisconsin

^h Smith top-cross derived from HATO fling synthetic

ⁱ Northwest Dent, open-pollinated variety once grown in northwest and north central U.S.

^j Synthetic from Mississippi

^k Composite of Southern U.S. prolific germplasm and Corn Belt lines made by W. L. Brown in the 1960's; known as "BS11" at Iowa State University

^l Hybrid once sold by Dockendorf

Materials and methods

DNA was extracted from 58 maize inbred lines (Table 1) and from four maize hybrids (Pioneer hybrids 3183, 3377, 3732, and 3747). The 58 inbreds encompass a broad range of genetic diversity for Corn Belt materials, including pairs of lines that span pedigree relationships from unrelated to highly related. Among these inbred lines were 13 sets of triplets (a progeny line and both its parents) that provided opportunities for tests of inheritance and/or reliable band scoring. In addition, four hybrids were also profiled, providing additional opportunities to check the scoring and inheritance of polymorphisms. Initial DNA extractions were made using the CTAB procedure (Saghai-Maroof et al. 1984). Subsequent DNA extractions were performed using a proprietary method for which patent protection is being sought. Both methods provide DNA suitable for amplification by these SSRs and gave equivalent results. SSR loci were individually amplified using DNA of each inbred and hybrid using protocols described by Chin et al. (1996), except that fluorescent-labeled primers were used. Samples containing 0.5 µl of the PCR products, 0.5 µl of GENESCAN 500 internal lane standard labeled with N, N, N', N'-tetramethyl-6-carboxyrhodamine (TAMARA) (Perkin Elmer-Applied Biosystems), and 50% formamide were heated at 92°C for 2 min, placed on ice, then loaded on 6% denaturing acrylamide gels. DNA samples were electrophoresed (29 W) for 7 h on an ABI Model 373A automatic DNA sequencer/fragment analyzer equipped with GENESCAN 672 software v. 1.2 (Perkin Elmer-Applied Biosystems). DNA fragments were sized automatically using the "local Southern" sizing algorithm (Elder and Southern 1987). PCR products from individual samples were assigned to specific alleles at each locus based on "binning" of a range of sizes (± 0.5 bp) as determined by ABI GeneScan™ and GENOTYPER™ software using the "local Southern" algorithm. Primer pairs for 200 potentially useful SSR loci were identified from the sequence data of maize that were published in Genbank, from di-repeat libraries made by Ben Burr (Brookhaven National Laboratory) and Lynn Senior (North Carolina State University), and from additional sequences available within Pioneer Hi-Bred Inter-

national, Inc. An initial screen of nine inbred lines was used to evaluate utility (Chin et al. 1996). Sequence data for primers to amplify these SSRs are available via the electronic maize database (Maize DB, Polacco 1996). Attempts were made to profile all of the 58 inbred lines and four hybrids with these SSRs. It was possible to obtain profiles for all of the inbreds and hybrids included in this survey for 131 SSRs (see Table 2). Genomic locations for most SSRs are provided according to the nomenclature used in Coc (1996). Among this set of SSRs, 59 (45%) were di-repeats, 36 (27%) were tri-repeats, 21 (16%) were tetra-repeats, 7 (5%) were penta-repeats, 5 (4%) were hexa-repeats, 2 (2%) were septa-repeats, and 1 (1%) was an octa-repeat.

RFLP data were obtained by Linkage Genetics (Salt Lake City, Utah) using DNA extraction and other protocols described by Helentjaris et al. (1985). Eighty single-locus probes that collectively sampled every chromosome arm were used.

PIC values were calculated using the algorithm:

$$PIC = 1 - \sum_{i=1}^n f_i^2 - 1,$$

where f_i^2 is the frequency of the i^{th} allele.

PIC provides an estimate of the discriminatory power of a locus by taking into account, not only the number of alleles that are expressed, but also the relative frequencies of those alleles. PIC values range from 0 (monomorphic) to 1 (very highly discriminative, with many alleles in equal frequencies). For example, a marker locus that reveals five alleles, but where one allele is found in very high frequency (e.g., freq. = 0.9), has overall less discriminatory capability than a locus that also has five alleles, but in which those alleles are found in more equal frequencies.

Genetic distances between pairs of inbred lines from SSR and RFLP data were calculated from comparisons of the band scores using a modified Nei's distance (Nei and Li 1979). Pedigree distances between pairs of inbreds were calculated from 1-Malecot's Coefficient of relatedness (Malecot 1948). Associations among inbreds from SSR, RFLP and pedigree data were revealed using average linkage cluster analysis.

Results

SSRs that failed to amplify against the majority of inbreds or which gave amplified products that could not be clearly resolved were re-amplified and electrophoresed a second time. If results were still poor, then primers were re-designed (designated with '-2' following the SSR locus name) for further evaluation. If amplified products still failed to yield clearly scorable profiles for less than 95% of the inbred lines, then those SSRs were discarded from this study. This exercise resulted in scorable data being obtained for the 58 inbreds and four hybrids from 131 SSRs (Table 2). Primers with different sequences for loci already published (Coe 1996) may result in amplification products with different molecular weights from those obtained using the initial primer sequences.

Thirteen parent-progeny triplets were available for the examination of inheritance and scoring accuracy. For SSRs, non-Mendelian scores (where an amplified product was scored in a progeny inbred that had not been scored in one or both parental inbreds) ranged from 0 to 7 of the SSRs (0–5.3% of SSRs) per triplet. The mean was 2.85 incidences of non-Mendelian scoring (2.2% of all SSRs) per triplet. For RFLPs the range of non-Mendelian scores was from 0 to 7 RFLPs per triplet (0–8.8% of RFLPs per triplet). The mean for RFLPs was 2.85 (3.6% of RFLPs) incidences of non-Mendelian scoring per triplet.

Twenty five of the 131 SSRs were associated with one or more incidences of non-Mendelian scoring in the triplets. One SSR (bngl 619), a di-repeat, was so detected in four triplets; phi 011, a tri-repeat resulted in non-Mendelian scores for three triplets; six SSRs gave rise to non-Mendelian scores in each of two triplets; the remaining 17 SSRs that gave rise to non-Mendelian scores did so in only single triplets. Of all the SSRs implicated in non-Mendelian scoring, ten were di-repeats (16% of all di-repeats), eight were tri-repeats (24% of all tri-repeats), five were tetra-repeats (24% of all tetra-repeats), and two penta-repeats (33% of all penta-repeats).

Incidences of non-Mendelian scoring (absence of a parental band in a hybrid or presence of a non-parental band in a hybrid) expressed as a percentage of the 131 SSR loci for each hybrid were 3% for Pioneer brand hybrids 3183 and 3377 and 1.5% for Pioneer brand hybrids 3732 and 3747. The mean was 2.3% per triplet. Of the 12 instances of non-Mendelian scoring that were found, 11 were due to the absence of one of the inbred parental bands in the hybrid and one resulted from the presence of a band in the hybrid that was scored in neither parent.

PIC values for SSRs are presented in Table 3. PIC values for SSRs ranged from 0.06 to 0.91; the mean PIC for SSRs was 0.62. Summary data for numbers of bands

and PIC values for each repeat class are presented in Table 4. Di-repeats gave high PIC values (0.70). Other frequently used classes (tri- and tetra-repeats) resulted in PIC values of 0.53 and 0.59, respectively.

Associations among inbreds on the basis of pedigree, RFLP and SSR data are presented in Figs. 1, 2 and 3, respectively. Associations of inbreds on the basis of pedigree (Fig. 1) were similar to that which could be expected on the basis of either marker method (Figs. 2 and 3). Very similar associations of inbreds were revealed from analyses of the RFLP and the SSR data (Figs. 2 and 3). The correlations of pairwise distances

Table 2 a SSR markers and map locations; primer sequences are given by Coe (1996)

SSR Locus	Genomic Location	SSR Locus	Genomic Location
phi056	1.01	bngl249	6.01
phi097	1.01	bngl107	6.02
bngl182	1.03	bngl480	6.03
bngl439	1.03	phi031	6.03
phi001	1.04	bngl176	6.04
bngl421	1.05	phi070	6.06
bngl615	1.07	phi025	6.07
bngl100	1.08	phi078	6.07
phi011	1.10	phi057	7.01
phi055	1.10	phi112	7.01
phi094	1.10	phi114	7.02
bngl504	1.11	bngl657	7.03
phi064	1.11	bngl434	7.03
bngl108	2.04	bngl155	7.04
bngl166	2.04	phi082	7.06
bngl420	2.04	bngl669	8.03
phi083	2.04	phi115	8.03
bngl602	3.04	phi119	8.03
nc030	3.04	bngl240	8.04
phi029	3.04	phi014	8.05
phi073	3.05	phi060	8.05
bngl197	3.07	phi015	8.08
phi072	4.01	phi080	8.08
phi021	4.02	phi017	9.02
bngl490	4.04	phi028	9.02
bngl667	4.04	phi033	9.02
bngl252	4.05	phi044	9.02
phi096	4.05	bngl127	9.03
phi092	4.08	bngl244	9.03
phi093	4.08	bngl430	9.03
bngl589	4.10	phi022	9.03
phi006	4.10	phi027	9.03
phi019	4.10	phi061	9.03
phi076	4.10	phi065	9.03
phi024	5.00	phi016	9.04
bngl143	5.01	phi042	9.04
bngl105	5.02	bngl128	9.07
phi113	5.02	bngl619	9.07
phi008	5.03	phi059	10.02
bngl653	5.04	phi063	10.02
bngl278	5.06	bngl640	10.03
bngl609	5.06	phi071	10.04
phi085	5.06	phi084	10.04
bngl386	5.09	bngl236	10.06
bngl238	6.00	bngl594	10.06
phi075	6.00		

No.

200200192



THE UNITED STATES OF AMERICA

TO ALL TO WHOM THESE PRESENTS SHALL COME:

Pioneer Hi-Bred International, Inc.

Whereas, THERE HAS BEEN PRESENTED TO THE

Secretary of Agriculture

AN APPLICATION REQUESTING A CERTIFICATE OF PROTECTION FOR AN ALLEGED DISTINCT VARIETY OF SEXUALLY REPRODUCED, OR TUBER PROPAGATED PLANT, THE NAME AND DESCRIPTION OF WHICH ARE CONTAINED IN THE APPLICATION AND EXHIBITS, A COPY OF WHICH IS HEREUNTO ANNEXED AND MADE A PART HEREOF, AND THE VARIOUS REQUIREMENTS OF LAW IN SUCH CASES MADE AND PROVIDED HAVE BEEN COMPLIED WITH, AND THE TITLE THERETO IS, FROM THE RECORDS OF THE PLANT VARIETY PROTECTION OFFICE, IN THE APPLICANT(S) INDICATED IN THE SAID COPY, AND WHEREAS, UPON DUE EXAMINATION MADE, THE SAID APPLICANT(S) IS (ARE) ADJUDGED TO BE ENTITLED TO A CERTIFICATE OF PLANT VARIETY PROTECTION UNDER THE LAW.

NOW, THEREFORE, THIS CERTIFICATE OF PLANT VARIETY PROTECTION IS TO GRANT UNTO THE SAID APPLICANT(S) AND THE SUCCESSORS, HEIRS OR ASSIGNS OF THE SAID APPLICANT(S) FOR THE TERM OF TWENTY YEARS FROM THE DATE OF THIS GRANT, SUBJECT TO THE PAYMENT OF THE REQUIRED FEES AND PERIODIC REPLENISHMENT OF VIABLE BASIC SEED OF THE VARIETY IN A PUBLIC REPOSITORY AS PROVIDED BY LAW, THE RIGHT TO EXCLUDE OTHERS FROM SELLING THE VARIETY, OR OFFERING IT FOR SALE, OR REPRODUCING IT, OR IMPORTING IT, OR EXPORTING IT, OR CONDITIONING IT FOR PROPAGATION, OR STOCKING IT FOR ANY OF THE ABOVE PURPOSE, OR CONDITIONING IT FOR PROPAGATION, OR STOCKING IT FOR ANY OF THE ABOVE PURPOSE, OR USING IT IN PRODUCING A HYBRID OR PLANT VARIETY THEREFROM, TO THE EXTENT PROVIDED BY THE PLANT VARIETY PROTECTION ACT. (84 STAT. 1505 AS AMENDED, 7 U.S.C. 2321 ET SEQ.)

CORN, FIELD

'PH94T'

In Testimony Whereof, I have hereunto set my hand and caused the seal of the Plant Variety Protection Office to be affixed at the City of Washington, D.C. this eighth day of July, in the year two thousand and four.

Attest:

R. M. J.

Commissioner
Plant Variety Protection Office
Agricultural Marketing Service

W. F. Foreman
Secretary of Agriculture

U.S. DEPARTMENT OF AGRICULTURE
AGRICULTURAL MARKETING SERVICE
SCIENCE AND TECHNOLOGY DIVISION - PLANT VARIETY PROTECTION OFFICE

APPLICATION FOR PLANT VARIETY PROTECTION CERTIFICATE
(Instructions and information collection burden statement on reverse)

The following statements are made in accordance with the Privacy Act of 1974 (5 U.S.C. 552a) and the Paperwork Reduction Act (PRA) of 1995.

Application is required in order to determine if a plant variety protection certificate is to be issued (7 U.S.C. 2421). Information is held confidential until certificate is issued (7 U.S.C. 2426).

1. NAME OF OWNER Pioneer Hi-Bred International, Inc.		2. TEMPORARY DESIGNATION OR EXPERIMENTAL NUMBER 		3. VARIETY NAME PH94T	
4. ADDRESS (Street and No. or RFD No., City, State and Zip Code, and Country) 7301 NW 62nd Avenue P.O. Box 85 Johnston, IA 50131-0085		5. TELEPHONE (include area code) 515/270-4051		<div style="border: 1px solid black; padding: 2px;"> FOR OFFICIAL USE ONLY PVPO NUMBER <div style="font-size: 1.5em; font-weight: bold;">200200192</div> </div>	
6. FAX (include area code) 515/253-2125		7. IF THE OWNER NAMED IS NOT A "PERSON", GIVE FORM OF ORGANIZATION (corporation, partnership, association, etc.) Corporation		8. IF INCORPORATED, GIVE STATE OF INCORPORATION IOWA	
9. DATE OF INCORPORATION March 5, 1999		10. NAME AND ADDRESS OF OWNER REPRESENTATIVE(S) TO SERVE IN THIS APPLICATION (FIRST PERSON LISTED WILL RECEIVE ALL PAPERS) Steven R. Anderson Research and Product Development P.O. Box 85 Johnston, IA 50131-0085		<div style="border: 1px solid black; padding: 2px;"> FILING & EXAMINATION FEES: <div style="font-size: 1.2em;">\$ 2705.00</div> DATE 6/6/02 CERTIFICATION FEE: <div style="font-size: 1.2em;">\$ 432.00</div> DATE 6/18/04 </div>	
11. TELEPHONE (include area code) 515/270-4051	12. FAX (include area code) 515/253-2125	13. E_MAIL <u>Steven.Anderson@Pioneer.com</u>		14. CROP KIND NAME (Common name) CORN	
15. GENUS AND SPECIES NAME OF CROP Zea Mays		16. FAMILY NAME (Botanical) Gramineae		17. IS THE VARIETY A FIRST GENERATION HYBRID? <input type="checkbox"/> Yes <input checked="" type="checkbox"/> No	
18. CHECK APPROPRIATE BOX FOR EACH ATTACHMENT SUBMITTED (Follow instructions on reverse) a. <input checked="" type="checkbox"/> Exhibit A. Origin and Breeding History of the Variety b. <input checked="" type="checkbox"/> Exhibit B. Statement of Distinctness c. <input checked="" type="checkbox"/> Exhibit C. Objective Description of the Variety d. <input type="checkbox"/> Exhibit D. Additional Description of the Variety (Optional) e. <input checked="" type="checkbox"/> Exhibit E. Statement of the Basis of the Owner's Ownership f. <input checked="" type="checkbox"/> Voucher Sample (2500 viable untreated seeds or, for tuber propagated varieties verification that tissue culture will be deposited and maintained in an approved public repository) g. <input checked="" type="checkbox"/> Filing and Examination Fee (\$2,705), made payable to "Treasurer of the United States" (Mail to Plant Variety Protection Office)			19. DOES THE OWNER SPECIFY THAT SEED OF THIS VARIETY BE SOLD AS A CLASS OF CERTIFIED SEED? See Section 83(a) of the Plant Variety Protection Act) <input type="checkbox"/> YES (If "yes", answer Items 20 and 21 below) <input checked="" type="checkbox"/> NO (If "no", go to Item 22)		
20. DOES THE OWNER SPECIFY THAT SEED OF THIS VARIETY BE LIMITED AS TO NUMBER OF CLASSES? <input type="checkbox"/> YES <input type="checkbox"/> NO IF "YES" WHICH CLASSES? <input type="checkbox"/> FOUNDATION <input type="checkbox"/> REGISTERED <input type="checkbox"/> CERTIFIED			21. DOES THE OWNER SPECIFY THAT SEED OF THIS VARIETY BE LIMITED AS TO NUMBER OF GENERATIONS? <input type="checkbox"/> YES <input type="checkbox"/> NO IF "YES" SPECIFY THE? <input type="checkbox"/> FOUNDATION <input type="checkbox"/> REGISTERED <input type="checkbox"/> CERTIFIED Number 1,2,3 etc. (If additional explanation is necessary, please use the space indicated on the reverse.)		
22. HAS THE VARIETY (INCLUDING ANY HARVESTED MATERIAL) OR A HYBRID PRODUCED FROM THIS VARIETY BEEN SOLD, DISPOSED OF, TRANSFERRED, OR USED IN THE U.S. OR OTHER COUNTRIES? <input type="checkbox"/> YES <input checked="" type="checkbox"/> NO IF YES, YOU MUST PROVIDE THE DATE OF FIRST SALE, DISPOSITION, TRANSFER, OR USE FOR EACH COUNTRY AND THE CIRCUMSTANCES. (Please use space indicated on reverse)			23. IS THE VARIETY OR ANY COMPONENT OF THE VARIETY PROTECTED BY INTELLECTUAL PROPERTY RIGHT (PLANT BREEDER'S RIGHT OR PATENT)? <input type="checkbox"/> YES <input checked="" type="checkbox"/> NO IF YES, PLEASE GIVE COUNTRY, DATE OF FILING OR ISSUANCE AND ASSIGNED REFERENCE NUMBER. (Please use space indicated on reverse.)		
24. The owner(s) declare that a viable sample of basic seed of the variety will be furnished with application and will be replenished upon request in accordance with such regulations as may be applicable, or for a tuber propagated variety a tissue culture will be deposited in a public repository and maintained for the duration of the certificate. The undersigned owner(s) is(are) the owner of this sexually reproduced or tuber propagated plant variety, and believe(s) that the variety is new, distinct, uniform, and stable as required in Section 42, and is entitled to protection under the provisions of Section 42 of the Plant Variety Protection Act. Owner(s) is(are) informed that false representation herein can jeopardize protection and results in penalties.					
SIGNATURE OF OWNER 			NAME (Please print or type) Steven R. Anderson		
NAME (Please print or type) 			DATE 5/30/2002		
CAPACITY OR TITLE 			DATE 		
			Research Scientist		

INSTRUCTIONS

200200192

GENERAL: To be effectively filed with the Plant Variety protection Office (PVPO), ALL of the following items must be received in the PVPO: (1) Completed application form signed by the owner; (2) completed Exhibits A, B, C, E; (3) for a seed reproduced variety at least 2,500 viable untreated seeds, for a hybrid variety at least 2,500 untreated seeds of each line necessary to reproduce the variety, or for tuber reproduced varieties verification that a viable (*in the sense that it will reproduce an entire plant*) tissue culture will be deposited and maintained in a approved public repository; (4) check drawn on a U.S. bank for \$2705 (\$320 filing fee and \$2,385 examination fee), payable to "Treasurer of the United States" (See Section 97.6 of the Regulations and Rules of Practice.) Partial applications will be held in the PVPO for not more than 90 days, then returned to the applicant as unfilled. Mail application and other requirements to Plant Variety Protection Office, AMS, USDA, Room 400, NAL Building, 10301 Baltimore Avenue, Beltsville, MD 20705-2351. Retain one copy for your files. All items on the face of the application are self explanatory unless noted below. Corrections on the application form and exhibits must be initialed and dated. DO NOT use masking materials to make corrections. If a certificate is allowed, you will be requested to send a check payable to "Treasurer of the United States" in the amount of \$320 for issuance of the certificate. Certificates will be issued to owner, not licensee or agent.

Plant Variety Protection Office

Telephone: (301)504-5518

FAX: (301)504-5291

Homepage: <http://www.ams.usda.gov/science/pvp.htm>

ITEM

- 18a. Give: (1) the genealogy, including public and commercial varieties, lines, or clones used, and the breeding method; (2) the details of subsequent stages of selection and multiplication; (3) evidence of uniformity and stability; and (4) the type and frequency of variants during reproduction and multiplication and state how these variants may be identified.
- 18b. Give a summary of the variety's distinctness. Clearly state how this application variety may be distinguished from all other varieties in the same crop. If the new variety is most similar to one variety or a group of related varieties: (1) identify these varieties and state all differences objectively; (2) attach statistical data for characters expressed numerically and demonstrate that these are clear differences; and (3) submit, if helpful, seed and plant specimens of photographs (prints) of seed and plant comparisons which clearly indicate distinctness.
- 18c. Exhibit C forms are available from the PVPO for most crops; specify crop kind. Fill in Exhibit C (Objective Description of Variety) form as completely as possible to describe your variety.
- 18d. Optional additional characteristics and/or photographs. Describe any additional characteristics that cannot be accurately conveyed in Exhibit C. Use comparative varieties as is necessary to reveal more accurately the characteristics that are difficult to describe, such as plant habit, plant disease resistance, etc.
- 18e. Section 52(5) of the Act required applicants to furnish a statement of the basis of the applicant's ownership. An Exhibit E form is available from the PVPO.
19. If "Yes" is specified (*seed of this variety be sold by variety name only, as a class of certified seed*), the applicant MAY NOT reverse this affirmative decision after the variety has been sold and so labeled, the decision published, or the certificate issued. However, if "No" has been specified, applicant may change the choice. (See Regulations and Rules of Practice, Section 7.103).
22. See Sections 41, 42, and 43 of the Act and Section 97.5 of the regulations for eligibility requirements.
23. See Section 5.5 of the Act for instructions on claiming the benefit of an earlier filing date
-
21. CONTINUED FROM FRONT (Please provide a statement as to the limitation and sequence of generations that may be certified.)
-
22. CONTINUED FROM FRONT (Please provide the date of first sale, disposition, transfer, or use for each country and the circumstances, if the variety (including any harvested material) or a hybrid produced from this variety has been sold, disposed of, transferred, or used in the U.S. or other countries.)
-
23. CONTINUED FROM FRONT (Please give the country, date of filing or issuance, and assigned reference number, if the variety or any component of the variety is protected by intellectual property right (Plant Breeder's Right or Patent).

NOTES: It is the responsibility of the applicant/owner to keep the PVPO informed of any changes of address or change of ownership or assignment or owner's representative during the life of the application/certificate. There is no charge for filing a change of address. The fee for filing a change of ownership or assignment or any modification of owner's name is specified in Section 97.175 of the regulations. (See Section 101 of the Act, and Sections 97.130, 97.131, 97.175(h) of Regulations and Rules of Practice.)

To avoid conflict with other variety names in use, the applicant should check the variety names proposed by contacting: Seed Branch, AMS, USDA, Room 213, Building 306, Beltsville Agricultural Research Center-East, Beltsville, MD 20705. Telephone: (301) 504-8089. <http://www.ams.usda.gov/lsg/seed/lsg-sd.htm>

According to the Paperwork Reduction Act of 1995, an agency may not conduct or sponsor, and a person is not required to respond to a collection of information unless it displays a valid OMB control number. The OMB control number for this collection of information is (0562-005). The time required to complete this information collection is estimated to average 1.4 hours per response, including the time for reviewing instructions, searching existing data sources, gathering and maintaining the data needed, and completing and reviewing the collection information.

The U.S. Department of Agriculture (USDA) prohibits discrimination in its programs on the basis of race, color, national origin, sex, religion, age, disability, political beliefs, and marital or familial status. (Not all prohibited bases apply to all programs). Persons with disabilities who require alternative means for communication of program information (braille, large print, audiotape, etc.) should contact the USDA Office of Communications at (202) 720-2791. To file a complaint, write the Secretary of Agriculture, U.S. Department of Agriculture, Washington, D.C. 20250, or call (202) 720-7327 (voice) or (202) 720-1127 (TDD). USDA is an equal employment opportunity employer.

Exhibit A. Origin and Breeding History

200200 192

Pedigree: PHME2/PH1K2)XB44K11K1

Pioneer Line PH94T, *Zea mays L.*, a dent corn inbred, was developed by Pioneer Hi-Bred International, Inc. from the single cross hybrid PHME2 X PH1K2 (PVP Certificate No. 9900376) using the pedigree method of plant breeding. Varieties PHME2 and PH1K2 are proprietary inbred lines of Pioneer Hi-Bred International, Inc. Selfing was practiced from the above hybrid for 8 generations using pedigree selection. During line development, crosses were made to inbred testers for the purpose of estimating the line's combining ability. Yield trials were grown at Parndorf, Austria, as well as other Pioneer research locations. After initial testing, additional hybrid combinations have been evaluated and subsequent generations of the line have been grown and hand-pollinated with observations again made for uniformity.

Variety PHME2 was derived by pedigree selection from the single cross hybrid PHR25 (PVP Certificate No. 8800002) X PHM10 (PVP Certificate No. 8900312).

Variety PH94T has shown uniformity and stability for all traits as described in Exhibit C - "Objective Description of Variety". It has been self-pollinated and ear-rowed 7 generations with careful attention paid to selection criteria and uniformity of plant type to assure genetic homozygosity and phenotypic stability. The line has been increased both by hand and in isolated fields with continued observations for uniformity and stability, and for 3 generations during the final stages of inbred development and seed multiplication. Very high standards for genetic purity have been established morphologically using field observations and electrophoretically using sound lab molecular marker methodology.

No variant traits have been observed or are expected in PH94T.

The criteria used in the selection of PH94T were yield, both per se and in hybrid combinations; late season plant health, grain quality, stalk lodging resistance, and kernel size, especially important in production. Other selection criteria include: ability to germinate in adverse conditions; disease and insect resistance; pollen yield and tassel size.

Season/Year Pedigree Grown	Inbreeding Level of Pedigree Grown
April/1995 PHME2	F0
April/1995 PH1K2	F0
Nov/1995 PHME2/PH1K2	F1
April/1996 PHME2/PH1K2)X	F2
April/1997 PHME2/PH1K2)XB4	F3
Nov/1997 PHME2/PH1K2)XB44	F4
April/1998 PHME2/PH1K2)XB44K1	F5
Nov/1998 PHME2/PH1K2)XB44K11	F6
April/1999 PHME2/PH1K2)XB44K11K1	F7
PHME2/PH1K2)XB44K11K1#	F8

*PH94T was selfed and ear-rowed from F3 through F8 generation.

#Uniformity and stability were established from F5 through F8 generation and beyond when seed supplies were increased.

Exhibit B: Novelty Statement

Variety PH94T mostly resembles Pioneer Hi-Bred International, Inc. proprietary inbred line PHTD5 (PVP Certificate No. 9400095). Tables 1A and 1B show two sample t-tests on data collected primarily in Johnston and Dallas Center, IA. The traits collectively show measurable differences between the two varieties.

Variety PH94T has more leaves above the top ear (6.8 vs 5.5) than variety PHTD5 (Table 1A, 1B).

Variety PH94T has a greater leaf width (9.5mm vs 7.5mm) than variety PHTD5 (Table 1A, 1B).

Variety PH94T has a greater ear diameter (41.6mm vs 35.5mm) than variety PHTD5 (Table 1A, 1B).

Variety PH94T has a greater ear weight (112.9g vs 69.3g) than variety PHTD5 (Table 1A, 1B).

Variety PH94T has a greater kernel length (10.7mm vs 9.1mm) than variety PHTD5 (Table 1A, 1B).

Variety PH94T has a greater cob diameter (25.1mm vs 21.7mm) than variety PHTD5 (Table 1A, 1B).

Exhibit B: Novelty Statement Tables

Table 1A: Data from Johnston and Dallas Center, IA broken out by 3 different locations in 2001 are supporting evidence for differences between PH94T and PHTD5. Locations had different environmental conditions. Environments had different planting dates and were in different fields. A two-sample t-test was used to compare differences between means.

cob diameter (mm)	AD	PH94T	PHTD5	5	5	24.4	20.8	3.6	1.140	1.304	0.510	0.583	8	4.6	0.002
cob diameter (mm)	DC	PH94T	PHTD5	5	5	26.4	21.2	5.2	0.894	0.447	0.400	0.200	8	11.6	0.000
cob diameter (mm)	JH	PH94T	PHTD5	5	5	24.4	23.2	1.2	1.140	0.837	0.510	0.374	8	1.9	0.094
ear diameter (mm)	AD	PH94T	PHTD5	5	5	42.4	35.6	6.8	2.191	1.817	0.980	0.812	8	5.3	0.001
ear diameter (mm)	DC	PH94T	PHTD5	5	5	42.2	34.2	8.0	0.447	2.280	0.200	1.020	8	7.7	0.000
ear diameter (mm)	JH	PH94T	PHTD5	5	5	40.2	36.6	3.6	1.643	2.074	0.735	0.927	8	3.0	0.016
ear weight (g)	AD	PH94T	PHTD5	5	5	124.8	81.4	43.4	5.404	15.339	2.417	6.860	8	6.0	0.000
ear weight (g)	DC	PH94T	PHTD5	5	5	120.8	58.6	62.2	12.795	17.869	5.722	7.991	8	6.3	0.000
ear weight (g)	JH	PH94T	PHTD5	5	5	93.0	67.8	25.2	9.823	14.856	4.393	6.644	8	3.2	0.013
kernel length (mm)	AD	PH94T	PHTD5	5	5	11.2	9.2	2.0	0.837	0.447	0.374	0.200	8	4.7	0.002
kernel length (mm)	DC	PH94T	PHTD5	5	5	11.0	9.8	1.2	0.000	0.447	0.000	0.200	8	6.0	0.000
kernel length (mm)	JH	PH94T	PHTD5	5	5	9.8	8.2	1.6	0.447	0.447	0.200	0.200	8	5.7	0.000
leaf number above top ear	AD	PH94T	PHTD5	5	5	7.0	5.6	1.4	0.707	0.548	0.316	0.245	8	3.5	0.008
leaf number above top ear	DC	PH94T	PHTD5	5	5	7.0	5.4	1.6	0.000	0.894	0.000	0.400	8	4.0	0.004
leaf number above top ear	JH	PH94T	PHTD5	5	5	6.4	5.4	1.0	0.894	0.548	0.400	0.245	8	2.1	0.066
leaf width (cm)	AD	PH94T	PHTD5	5	5	10.4	8.2	2.2	0.548	0.447	0.245	0.200	8	7.0	0.000
leaf width (cm)	DC	PH94T	PHTD5	5	5	9.0	6.4	2.6	0.000	0.548	0.000	0.245	8	10.6	0.000
leaf width (cm)	JH	PH94T	PHTD5	5	5	9.2	8.0	1.2	0.447	0.707	0.200	0.316	8	3.2	0.012

Exhibit B. Novelty Statement Tables

Table 1B: Summary data from Johnston and Dallas Center, IA across environments in 2001 are supporting evidence for differences between PH94T and PHTD5. Environments had different planting dates and were in different fields. A two-sample t-test was used to compare differences between means.

	leaf number above top ear	PH94T	PHTD5	15	15	6.8	5.5	1.3	0.676	0.640	0.175	0.165	28	5.5	0.000
leaf width (cm)		PH94T	PHTD5	15	15	9.5	7.5	2.0	0.743	0.990	0.192	0.256	28	6.3	0.000
ear diameter (mm)		PH94T	PHTD5	15	15	41.6	35.5	6.1	1.805	2.167	0.466	0.559	28	8.4	0.000
ear weight (g)		PH94T	PHTD5	15	15	112.9	69.3	43.6	17.233	17.762	4.450	4.586	28	6.8	0.000
kernel length (mm)		PH94T	PHTD5	15	15	10.7	9.1	1.6	0.816	0.799	0.211	0.206	28	5.4	0.000
cob diameter (mm)		PH94T	PHTD5	15	15	25.1	21.7	3.3	1.387	1.387	0.358	0.358	28	6.6	0.000

200200 192

United States Department of Agriculture, Agricultural Marketing Service
Science Division, Plant Variety Protection Office
National Agricultural Library Building, Room 500
Beltsville, MD 20705

Objective Description of Variety
Corn (*Zea mays* L.)

Name of Applicant (s) Pioneer Hi-Bred International, Inc.		Variety Seed Source	Variety Name or Temporary Designation PH94T	
Address (Street & No., or RFD No., City, State, Zip Code and Country) 7301 NW 62nd Avenue, P.O. Box 85, Johnston, Iowa 50131-0085			FOR OFFICIAL USE	200200 192
			PVP0 Number	
Place the appropriate number that describes the varietal characters typical of this inbred variety in the spaces below. Right justify whole numbers by adding leading zeroes if necessary. Completeness should be striven for to establish an adequate variety description. Traits designated by an "*" are considered necessary for an adequate variety description and must be completed.				
COLOR CHOICES (Use in conjunction with Munsell color code to describe all color choices: describe #25 and #26 in Comments section):				
01=Light Green	06=Pale Yellow	11=Pink	16=Pale Purple	21=Buff
02=Medium Green	07=Yellow	12=Light Red	17=Purple	22=Tan
03=Dark Green	08=Yellow Orange	13=Cherry Red	18=Colorless	23=Brown
04=Very Dark Green	09=Salmon	14=Red	19=White	24=Bronze
05=Green-Yellow	10=Pink-Orange	15=Red & White	20=White Capped	25=Variegated (Describe)
				26=Other (Describe)
STANDARD INBRED CHOICES				
(Use the most similar (in background and maturity) of these to make comparisons based on grow-out trial data):				
Yellow Dent Families:		Yellow Dent (Unrelated):	Sweet Corn:	
Family	Members	Co109, ND246,	C13, Iowa5125, P39, 2132	
B14	CM105, A632, B64, B68	Oh7, T232,		
B37	B37, B76, H84	W117, W153R,	Popcorn:	
B73	N192, A679, B73, NC268	W18BN	SG1533, 4722, HP301, HP7211	
C103	Mo17, Va102, Va35, A682			
Oh43	A619, MS71, H99, Va26	White Dent:	Pipcorn:	
WP9	W64A, A554, A654, Pa91	C166, H105, Ky228	Mo15W, Mo16W, Mo24W	

Groups on Lynn/Osborn/Grimes/98-99PVP

EXHIBIT C: PH94T

1. TYPE: (describe intermediate types in Comments section):				Standard Variety Name	
2 1=Sweet 2=Dent 3=Flint 4=Flour 5=Pop 6=Ornamental <u>Dent</u>				<u>A554</u>	
2. REGION WHERE DEVELOPED IN THE U.S.A.:				Standard Seed Source	
1 1=Northwest 2=Northcentral 3=Northeast 4=Southeast 5=Southcentral 6=Southwest 7=Other				<u>AMES 19305</u>	
3. MATURITY (In Region of Best Adaptability; show Heat Unit formula in 'Comments' section)				DAYS HEAT UNITS	
DAYS HEAT UNITS				DAYS HEAT UNITS	
<u>064</u> <u>1,259.0</u> From emergence to 50% of plants in silk				<u>065</u> <u>1,274.7</u>	
<u>063</u> <u>1,243.0</u> From emergence to 50% of plants in pollen				<u>064</u> <u>1,256.7</u>	
<u>002</u> <u>0,046.7</u> From 10% to 90% pollen shed				<u>002</u> <u>0,065.0</u>	
From 50% silk to optimum edible quality					
From 50% silk to harvest at 25% moisture					
4. PLANT:				Standard Sample	
				Deviation Size	
<u>194.3</u> cm Plant Height (to tassel tip)				<u>166.7</u> <u>10.97</u> <u>03</u>	
<u>077.0</u> cm Ear Height (to base of top ear node)				<u>051.0</u> <u>03.00</u> <u>03</u>	
<u>013.7</u> cm Length of Top Ear Internode				<u>012.2</u> <u>01.11</u> <u>03</u>	
<u>0.0</u> Average Number of Tillers/plant				<u>0.0</u> <u>00.01</u> <u>03</u>	
<u>1.1</u> Average Number of Ears per Stalk				<u>0.9</u> <u>00.06</u> <u>03</u>	
<u>1</u> Anthocyanin of Brace Roots: 1=Absent 2=Faint 3=Moderate 4=Dak 5=Very Dark				<u>3</u>	
5. LEAF:				Standard Sample	
				Deviation Size	
<u>09.5</u> cm Width of Ear Node Leaf				<u>08.5</u> <u>00.50</u> <u>03</u>	
<u>75.0</u> cm Length of Ear Node Leaf				<u>67.1</u> <u>05.60</u> <u>03</u>	
<u>07</u> Number of leaves above top ear				<u>06</u> <u>00.20</u> <u>03</u>	
<u>26</u> Degrees Leaf Angle (measure from 2nd leaf above ear at anthesis to stalk above leaf)				<u>30</u> <u>04.33</u> <u>03</u>	
<u>03</u> Leaf Color (Munsell code) <u>7.5GY3.4</u>				<u>03</u> <u>5GY4.4</u>	
<u>1</u> Leaf Sheath Pubescence (Rate on scale from 1=none to 9=like peach fuzz)				<u>2</u>	
Marginal Waves (Rate on scale from 1=none to 9=many)					
Longitudinal Creases (Rate on scale from 1=none to 9=many)					
6. TASSEL:				Standard Sample	
				Deviation Size	
<u>12</u> Number of Primary Lateral Branches				<u>10</u> <u>03.34</u> <u>03</u>	
<u>49</u> Branch Angle from Central Spike				<u>30</u> <u>07.57</u> <u>03</u>	
<u>51.0</u> cm Tassel Length (from top leaf collar node to tassel tip)				<u>50.3</u> <u>01.60</u> <u>03</u>	
<u>5</u> Pollen Shed (rate on scale from 0=male sterile to 9=heavy shed)				<u>5</u>	
<u>07</u> Anther Color (Munsell code) <u>10Y9.6</u>				<u>07</u> <u>5Y8.8</u>	
<u>01</u> Glume Color (Munsell code) <u>7.5GY5.6</u>				<u>01</u> <u>5GY6.6</u>	
<u>1</u> Bar Glumes (Glume Bands): 1=Absent 2=Present				<u>1</u>	
Application Variety Data				Standard Variety Data	
Page 1					

JMS 9/30/03

Application Variety Data

PH94T

Page 2

Standard Variety Data

7a. EAR (Unhusked Data):

01	Silk Color (3 days after emergence) (Munsell code)	2.5GY66	11	10RP56
01	Fresh Husk Color (25 days after 50% silking) (Munsell code)	5GY68	01	5GY78
21	Dry Husk Color (65 days after 50% silking) (Munsell code)	2.5Y84	21	2.5Y8.54
2	Position of Ear at Dry Husk Stage: 1= Upright 2= Horizontal 3= Pendant		3	
6	Husk Tightness (Rate of Scale from 1=very loose to 9=very tight)		6	
2	Husk Extension (at harvest): 1=Short (ears exposed) 2=Medium (<8 cm) 3=Long (8-10 cm beyond ear tip) 4=Very Long (>10 cm)		2	

7b. EAR (Husked Ear Data):

	Standard	Sample	Standard	Sample
	Deviation	Size	Deviation	Size
15.0 cm Ear Length	01.00	03	09.0	01.00 03
41.3 mm Ear Diameter at mid-point	01.15	03	37.0	02.65 03
113.0 gm Ear Weight	17.44	03	57.7	18.23 03
16 Number of Kernel Rows	00.58	03	14.3	00.58 03
2 Kernel Rows: 1=Indistinct 2=Distinct			2	
2 Row Alignment: 1=Straight 2=Slightly Curved 3=Spiral			2	
15.7 cm Shank Length	00.58	03	07.3	03.51 03
2 Ear Taper: 1=Slight 2= Average 3=Extreme			2	

8. KERNEL (Dried)

	Standard	Sample	Standard	Sample
	Deviation	Size	Deviation	Size
10.7 mm Kernel Length	00.58	03	09.7	01.15 03
07.0 mm Kernel Width	00.00	03	07.0	00.00 03
05.3 mm Kernel Thickness	00.58	03	04.3	00.58 03
44.7 % Round Kernels (Shape Grade)	08.62	03	25.7	12.58 03
1 Aleurone Color Pattern: 1-Homozygous 2=Segregating			1	
08 Aleurone Color (Munsell code)	7.5YR7/6		07	2.5YR7/2
07 14 Hard Endosperm Color (Munsell code)	10YR6/2		07	10YR7/2
03 Endosperm Type:			3	
1=Sweet (Su1) 2=Extra Sweet (sh2) 3=Normal Starch				
4=High Amylose Starch 5=Waxy Starch 6=High Protein				
7=High Lysine 8=Super Sweet (se) 9=High Oil				
10=Other_				
25.3 gm Weight per 100 Kernels (unsized sample)	01.53	03	18.00	04.36 03

9. COB:

	Standard	Sample	Standard	Sample
	Deviation	Size	Deviation	Size
24.7 mm Cob Diameter at mid-point	01.15	03	22.0	02.65 03
14 Cob Color (Munsell code)	2.5YR4/8		14	10R4/8

Application Variety Data

Page 2

Standard Variety Data

JMS 4/21/04

10. DISEASE RESISTANCE (Rate from 1 (most susceptible) to 9 (most resistant);
leave blank if not tested; leave Race or Strain Options blank if polygenic):

A. Leaf Blights, Wilts, and Local Infection Diseases

	Anthracnose Leaf Blight (<i>Colletotrichum graminicola</i>)	
6	Common Rust (<i>Puccinia sorghi</i>)	5
	Common Smut (<i>Ustilago maydis</i>)	
	Eyespot (<i>Kabatiella zeae</i>)	
	Goss's Wilt (<i>Clavibacter michiganense</i> spp. <i>nebraskense</i>)	
	Gray Leaf Spot (<i>Cercospora zeae-maydis</i>)	
	Helminthosporium Leaf Spot (<i>Bipolaris zeicola</i>) Race _____	
	Northern Leaf Blight (<i>Exserohilum turcicum</i>) Race _____	
	Southern Leaf Blight (<i>Bipolaris maydis</i>) Race _____	
	Southern Rust (<i>Puccinia polysora</i>)	
7	Stewart's Wilt (<i>Erwinia stewartii</i>)	7
	Other (Specify) _____	

B. Systemic Diseases

	Corn Lethal Necrosis (MCMV and MDMV)	
9	Head Smut (<i>Sphacelotheca reiliana</i>)	9
	Maize Chlorotic Dwarf Virus (MDV)	
	Maize Chlorotic Mottle Virus (MCMV)	
	Maize Dwarf Mosaic Virus (MDMV)	
	Sorghum Downy Mildew of Corn (<i>Peronosclerospora sorghi</i>)	
	Other (Specify) _____	

C. Stalk Rots

9	Anthracnose Stalk Rot (<i>Colletotrichum graminicola</i>)	5
	Diplodia Stalk Rot (<i>Stenocarpella maydis</i>)	
	Fusarium Stalk Rot (<i>Fusarium moniliforme</i>)	
	Gibberella Stalk Rot (<i>Gibberella zeae</i>)	
	Other (Specify) _____	

D. Ear and Kernel Rots

	Aspergillus Ear and Kernel Rot (<i>Aspergillus flavus</i>)	
	Diplodia Ear Rot (<i>Stenocarpella maydis</i>)	
6	Fusarium Ear and Kernel Rot (<i>Fusarium moniliforme</i>)	9
6	Gibberella Ear Rot (<i>Gibberella zeae</i>)	6
	Other (Specify) _____	

11. INSECT RESISTANCE (Rate from 1 (most susceptible) to 9 (most resistant); (leave blank if not tested) :

Banks grass Mite (*Oligonychus pratensis*)
 Corn Worm (*Helicoverpa zea*)
 Leaf Feeding
 Silk Feeding
 mg larval wt.
 Ear Damage
 Corn Leaf Aphid (*Rhopalosiphum maidis*)
 Corn Sap Beetle (*Carpophilus dimidiatus*)
 European Corn Borer (*Ostrinia nubilalis*)
 1st Generation (Typically Whorl Leaf Feeding)
 2nd Generation (Typically Leaf Sheath-Collar Feeding)
 Stalk Tunneling
 cm tunneled/plant
 Fall Armyworm (*Spodoptera frugiperda*)
 Leaf Feeding
 Silk Feeding
 mg larval wt.
 Maize Weevil (*Sitophilus zeamais*)
 Northern Rootworm (*Diabrotica barberi*)
 Southern Rootworm (*Diabrotica undecimpunctata*)
 Southwestern Corn Borer (*Diatraea grandiosella*)
 Leaf Feeding
 Stalk Tunneling
 cm tunneled/plant
 Two-spotted Spider Mite (*Tetranychus urticae*)
 Western Rootworm (*Diabrotica virgifera virgifera*)
 Other (Specify) _____

12. AGRONOMIC TRAITS:

5	Staygreen (at 65 days after anthesis) (Rate on a scale from 1=worst to excellent)	4
0.0	% Dropped Ears (at 65 days after anthesis)	0.0
	% Pre-anthesis Brittle Snapping	
	% Pre-anthesis Root Lodging	
0.0	Post-anthesis Root Lodging (at 65 days after anthesis)	50.6
4,324.0	Kg/ha Yield of Inbred Per Se (at 12-13% grain moisture)	1,710.8

13. MOLECULAR MARKERS: (0=data unavailable; 1=data available but not supplied; 2=data supplied):

1 Isozymes

0 RFLP's

0 RAPD's

COMMENTS (eg. state how heat units were calculated, standard inbred seed source, and/or where data was collected. Continue in Exhibit D):

CLARIFICATION OF DATA IN EXHIBITS B AND C

Please note the data presented in Exhibit B and C, "Objective Description of Variety," are collected primarily at Johnston and Dallas Center, Iowa. The data in Tables 1A and 1B are from two sample t-tests using data collected in Johnston and Dallas Center, IA. These traits in exhibit B collectively show distinct differences between the two varieties.

JMS 9/30/03

The data collected in exhibit C was collected in 2001 for page 1 and 2. There were 3 different planting dates planted for these trials. There are environmental factors that differ from planting date to planting date. Environmental temperature and precipitation differences during the vegetative and grain fill periods can impact plant and grain traits, and are a source of variability. The environmental conditions described above could result in larger standard deviations. The variation associated with environment to environment is normally higher than the variation associated within locations. Also, the ear and sizing traits can vary depending on how well pollinated the ears are and how consistent the weather is during the grain fill period. I have enclosed a table that shows monthly temperature and precipitation in 2001.

200200192

C
Exhibit D. Temperature and Precipitation differences from Ankeny, IA

TEMPERATURE

YEAR	MAY	JUN	JULY	AUG	AVERAGE
1994	59.8	70.7	71.9	69.0	67.9
1995	56.2	69.4	74.3	76.9	69.2
1996	56.2	69.3	71.3	70.5	66.8
1997	53.5	70.6	74.1	69.6	67.0
1998	64.7	66.6	74.8	73.5	69.9
1999	60.7	69.7	78.7	70.5	69.9
2000	63.5	68.9	73.2	74.2	70.0
2001	61.3	69.0	76.7	74.2	70.3
2002	57.7	73.5	77.9	71.7	70.2

RAINFALL

YEAR	MAY	JUN	JULY	AUG	Total
1994	3.67	5.75	1.71	4.18	15.31
1995	5.04	4.19	2.94	2.87	15.04
1996	8.47	4.35	2.51	2.14	17.47
1997	4.32	3.27	4.10	1.36	13.05
1998	6.46	11.07	5.70	4.96	28.19
1999	6.46	4.54	4.45	6.55	21.85
2000	5.40	5.80	3.16	1.78	16.14
2001	5.72	3.87	2.05	1.92	13.56
2002	2.91	2.78	5.34	4.00	15.03

EXHIBIT E
STATEMENT OF THE BASIS OF OWNERSHIP

Application is required in order to determine if a plant variety protection certificate is to be issued (7 U.S.C. 2421). Information is held confidential until certificate is issued (7 U.S.C. 2426).

1. NAME OF APPLICANT(S) PIONEER HI-BRED INTERNATIONAL, INC.	2. TEMPORARY DESIGNATION OR EXPERIMENTAL NUMBER	3. VARIETY NAME PH94T
4. ADDRESS (Street and No., or R.F.D. No., City, State, and ZIP, and Country) 7301 NW 62nd AVENUE P.O.BOX 85 JOHNSTON, IA 50131-0085	5. TELEPHONE (Include area code) 515-270-4051	6. FAX (include area code) 515-253-2125
7. PVPO NUMBER 200200 192		
8. Does the applicant own all rights to the variety? Mark an "X" in appropriate block. If no, please explain: <input checked="" type="checkbox"/> YES <input type="checkbox"/> NO		

9. Is the applicant (individual or company) a U.S. national or U.S. based company? <input checked="" type="checkbox"/> YES <input type="checkbox"/> NO If no, give name of country
10. Is the applicant the original owner? <input checked="" type="checkbox"/> YES <input type="checkbox"/> NO If no, please answer <u>one</u> of the following: a. If original rights to variety were owned by individual(s), is(are) the original owner(s) a U.S. national(s)? <input type="checkbox"/> YES <input type="checkbox"/> NO If no, give name of country b. If original rights to variety were owned by a company(ies), is(are) the original owner(s) a U.S. based company? <input checked="" type="checkbox"/> YES <input type="checkbox"/> NO If no, give name of country

11. Additional explanation on ownership (If needed, use reverse for extra space): PH94T is owned by Pioneer Hi-Bred International, Inc.

Pioneer Hi-Bred International, Inc. (PHI), Des Moines, Iowa, and/or its wholly owned subsidiary Pioneer Overseas Corporation (POC), Des Moines, Iowa, is the employer of the plant breeders involved in the selection and development of PH94T. Pioneer Hi-Bred International and/or Pioneer Overseas Corporation has the sole rights and ownership of PH94T pursuant to written contracts that assign all rights in the variety to PHI and/or POC at the time such variety was created. No rights to this variety are retained by any individuals.

PLEASE NOTE:

Plant variety protection can be afforded only to owners (not licensees) who meet one of the following criteria:

1. If the rights to the variety are owned by the original breeder, that person must be a U.S. national, national of a UPOV member country, or national of a country Which affords similar protection to nationals of the U.S. for the same genus and species.
2. If the rights to the variety are owned by the company which employed the original breeder(s), the company must be U.S. based, owned by nationals of a UPOV member country, or owned by national of a country which affords similar protection to nationals of the U.S. for the same genus and species.
3. If the applicant is an owner who is not the original owner, both the original owner and the applicant must meet one of the above criteria.

The original breeder/owner may be the individual or company who directed final breeding. See section 41(a)(2) of the Plant Variety Protection Act for definition.

According to the Paperwork Reduction Act of 1995, no persons are required to respond to a collection of information unless it displays a valid OMB control number. The valid OMB control number for this information collection is 0581-0055. The time required to complete this information collection is estimated to average 10 minutes per response, including the time for reviewing instructions, searching existing data sources, gathering and maintaining the data needed, and completing and reviewing the collection of information.

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